REPORT

MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
PHASE III: EFFECTS OF LIFE-TIME EXPOSURE
PART II: TRINITROGLYCERIN

PROGRESS REPORT NO. 8

November 1978

Contract No. DAMD-17-74-C-4073
MRI Project No. 3900-B

For

Contract Officer's Technical Representative: Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

Animal Experimentation: Animal experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1974) prepared by the Institute of Laboratory Animal Resources, National Research Council: the regulations and standards prepared by the Department of Agriculture; and Public Law 91-570, "Laboratory Animal Welfare Act," 1970.

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Fort Detrick, Frederick, MD 21701

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Trinitroglycerin	Reproductive Toxicity	Risk Assessment
Chronic Toxicity	Mutagenesis	Water Quality Criterion
Carcinogenesis	Metabolism	
Nitroglycerin	1,2,3-Propanetriol Trinitrate (CAS Reg. No. 55-63-0)	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The effects of oral doses of trinitroglycerin (TNG) after oral administration for up to 24 months were studied in dogs, rats and mice. Ancillary studies included cytogenetic analysis, three generation reproduction, dominant lethal mutation and metabolism studies in rats.

In dogs, 1, 5, or 25 mg/kg/day by capsule for 12 months produced a little, dose-related, transient methemoglobinemia. In rats, 3.04 or 3.99 mg/kg/day in feed for males or females, respectively, had no apparent effects, 31.5 or

20 (concluded)

8.1 mg/kg/day had some hepatotoxic effects and 363 or 434 mg/kg/day was highly toxic, but not lethal. In mice, up to 115 or 96 mg/kg/day had no effect, while 1,020 or 1,060 mg/kg/day was toxic.

TNG caused some non-specific effects on weight gain, feed consumption and behavior. Target organs included the blood (methemoglobinemia with compensated anemia and pigment deposits in rodents), liver (cholangiofibrosis and hepatocellular carcinoma in rats) and testis (increased interstitial tissue and interstitial cell tumors in rats). No direct effects were seen in the ancillary studies.

From these data the concentration of TNG in ambient water which would produce in man a risk of 1 in 100,000 of developing a tumor after lifetime exposure was estimated as 28.9 ng/liter.

microgram

FOREWORD

The U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL), Fort Detrick, Frederick, MD, has been conducting a research program since 1973 for the purpose of developing the scientific data base from which water quality criteria for compounds unique to the munitions industry could be determined. A water quality criterion (as defined by the amended Clean Water Act, 1977) is a qualitative or quantitative estimate of the concentration of a pollutant in ambient waters that, when not exceeded, will ensure a water quality sufficient to protect a specified water use. The criterion is a scientific entity based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility. Currently, a water quality criterion consists of two separate numerical limits, one for the protection of human health and the other for the protection of aquatic organisms. These numbers, when translated by the appropriate regulatory agency, can be the basis of enforceable discharge or effluent limitations in a point source discharge permit issued under the Clean Water Act.

Since a water quality criterion is to protect designated water uses, a diverse, multidisciplined research program was developed by USAMBRDL that includes "effects" studies on laboratory and domestic animals, wildlife species, aquatic organisms, plants, and economically important crops. In addition, extensive chemical and biological fate and persistence tests are conducted to provide information on the behavior of a pollutant in the aqueous environment. These kinds of data are especially useful for making site-specific translation of criteria into enforceable discharge limits.

This report represents a portion of the mammalian toxicology data base being developed by USAMBRDL on materials related to the use and disposal of trinitroglycerin.

PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD-17-74-C-4073, MRI Project No. 3900-B, "Munition Compounds Mammalian Toxicity Study." The work was supported by the U.S. Army Medical Research and Development Command, Department of the Army. Dr. Jack C. Dacre, Environmental Protection Research Division, USAMBRDL, was the contract officer's technical representative for the project.

This work was conducted in the Biological Sciences Division under the direction of Dr. William B. House, between October 1, 1975 and March 31, 1978, and Dr. Harold M. Hubbard, between April 1 and September 30, 1978. The experimental work was directed by Dr. Cheng-Chun Lee, Deputy Director, with the assistance of Dr. Harry V. Ellis, III, Senior Pharmacologist. Mr. Jack H. Hagensen, Supervisor, supervised the animal experimentation with the technical assistance of Karen J. Smith, E. Renee Walton, Darrell L. Lavish, Pam J. Saunders, Linda J. Ryhal and J. Christopher Unger. Dr. John R. Hodgson, Head, Biochemical and Developmental Pharmacology, supervised the studies on metabolism, cytogenesis and mutagenesis, with technical assistance of Daniel L. VanGoethem, Mary A. Kowalski, Maxine Hainje and Rita D. Freeman. Mr. Jan L. Minor, Assistant Toxicologist, supervised reproduction studies and the computer program and analysis of experimental data, with technical assistance of Timothy M. Unger. Dr. Danny O. Helton, Senior Chemist, performed the TNG assay in feed. Dr. C. B. Hong, Senior Veterinary Pathologist, supervised the necropsy and the histology preparation and with Dr. Helmuth Sprinz, Consulting Pathologist, performed the microscopic examination, with technical assistance of Ellen R. Ellis, Kerry L. Crabb, Janet Kliethermes, Ernesto A. Castillo, Judith Shifrin, and Hung D. Hoang. Miss Judith D. Girvin (ASCP certified M.T.), Labortory Supervisor, supervised the hematology and clinical laboratory tests, with the technical assistance of Ilonna S. Elwood, Duane R. Smith and Bhanu S. Gosalia. Dr. Betty L. Herndon, Associate Pharmacologist, prepared the water quality criteria.

Approved for:

MIDWEST RESEARCH INSTITUTE

Florence I. Metz, Ph.D.
Acting Director
Pharmacology/Toxicology

December 21, 1979

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EXECUTIVE SUMMARY

The effects of trintitroglycerin (TNG) after oral dosing for up to 2 years were studied in dogs, rats and mice. Ancillary studies included cytogenetics analysis of kidney and bone marrow cells cultured from dogs and rats, a dominant lethal mutation study in rats, a three generation reproduction study in rats and metabolism studies in rats fed TNG chronically.

Dogs were given daily doses of 0, 1, 3 or 25 mg/kg of TNG in capsules for 1 year. Periodic blood tests found an occasional dose-related incidence of transient methemoglobinemia. This small amount of methemoglobin is readily corrected by the body, because we saw no accompanying effects on body weight, feed consumption, other hematology tests, clincial chemistry and histopathological examination.

In rats, the low dose produced TNG intakes of 3.04 mg/kg/day and 3.99 mg/kg/day in males and females, respectively, but no apparent toxic effects in the 24 month study. The middle dose, 31.5 and 38.1 mg/kg/day, respectively, caused some hepatic lesions (areas or foci of hepatocellular alteration). The high dose, 363 and 434 mg/kg/day, respectively, caused many adverse effects, including decreased feed consumption, depressed weight gain, behavioral effects (decreased activity, failure to groom), and methemoglobinemia, with some excessive pigmentation in the spleens and the renal epithelium. In the liver, the lesions seen in middle dose rats had developed through the stage of neoplastic nodules to hepatocellular carcinomas, some of which metastasized to the lung. In addition, the livers were extremely large, primarily from extensive cholangiofibrosis. Interstitial cell tumors in some high dose males caused aspermatogenesis. One beneficial effect, a decrease in the naturally occurring incidence of the most common tumors in this strain of rats (pituitary chromophobe adenoma and mammary fibroadenoma), contributed to an increased lifespan in these high dose rats, especially the females.

In mice, no adverse effects were seen with the low (11.1 mg/kg/day TNG intake in males and 9.7 mg/kg/day in females) and middle (115 and 96 mg/kg/day, respectively) doses during the 24 month study. The high dose (1,020 and 1,060 mg/kg/day, respectively) caused lower feed consumption and weight gain, behavioral effects (decreased activity, failure to groom) and methemoglobinemia and sequelae (Heinz bodies, reticulocytosis, and deposits of pigment in the liver and some other organs).

There were no apparent TNG-induced mutagenic effects in the cytogenetics analyses of cells from dogs and rats and in the dominant lethal mutation study in rats.

The three generation reproduction study found adverse effects in the high dose rats. These were the result of the decreased feed intake and consequent poor nutritional status of the females and decreased spermatogenesis (accompanied by increased interstitial tissue) in the males. There was no specific teratologic effect or other specifically reproductive effects.

The results of metabolism studies in rats fed TNG for 3, 12 or 24 months were substantially the same as those in rats not fed TNG, despite the massive liver toxicity in the TNG-fed rats. TNG was well absorbed, widely distributed and concentrated only in liver. TNG was rapidly denitrated toward glycerin. The partially nitrated compounds were glucuronidated and excreted in the urine. The glycerin was excreted in the urine, oxidized to CO_2, or converted into a variety of other metabolites.

It is significant that the adverse effect of TNG was noted only at doses vastly larger than the maximum recommended human dose - 0.14 mg/kg/day.

Because TNG has carcinogenic effects, an ambient water concentration of zero is necessary for maximum protection of human health. However, using EPA developed methodology, exposure to 2$\mathcal{E}$.9 µg/liter of TNG for a lifetime produces an estimated risk of 10^{-5} (one in 100,000) that a tumor will develop in man. A ten-fold decrease in dose would produce a ten-fold decrease in the estimated risk.

I. INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munition Compounds Mammalian Toxicity Study," we have performed a variety of studies, divided into three phases. Phase I, Effects of Acute Exposure, includes acute oral toxicitity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism studies. Results were reported in Progress Report No. 1.[1] Results on additional compounds plus in vitro mutagenic (Ames test) studies were submitted as Report No. 6.[2] Phase II, Effects of Multiple Exposure, includes subacute and subchronic toxicity, reversibility, immunologic response, chemical-biological interaction, mutagenicity, and disposition and metabolism studies. Results were presented in a series of reports on the compounds tested, trinitroglycerin (TNG),[3] 2,4-dinitrotoluene (2,4-DNT),[4] 2,6-dinitrotoluene,[5] and nitrocellulose (NC).[6] Phase III, Effects of Life-Time Exposure, includes chronic toxicity, reversibility, reproductive, cytogenetic, and metabolism studies on three of those compounds, 2,4-DNT, TNG and NC. This report contains the results of studies on TNG.

II. MATERIALS AND METHODS

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II. MATERIALS AND METHODS

Materials and methods employed in these studies are described below.

A. Animals

1. Sources

Young, healthy beagles were bought from Hazleton Research Animals (Cumberland, Virginia). Young healthy CD® rats and CD-1® mice were bought from Charles River Breeding Laboratory (Wilmington, Massachusetts). All animals were maturing. They were conditioned in our animal quarters for at least 2 weeks.

2. Housing and Animal Husbandry

Dogs were kept in dog pens with outside runs. Up to 12 dogs shared 60 sq ft of heated inside space and 120 sq ft of outside space. Water was available continuously. Dogs were fed ad libitum or as described below under feed measurement. Runs were cleaned daily.

Rats and mice were kept in plastic cages with hardwood chip bedding, metal lids and filter tops. Bedding was steam-sterilized before use and changed at least weekly. Cages, tops and water bottles were steam-sterilized before use and changed weekly. Feed and water were available at all times. Usually two male rats, three female rats, four male mice, or four female mice were housed in each cage and differentiated by ear-punches. Some groups (especially male mice) were subdivided to prevent fighting. The rodent quarters are fully air conditioned, with 10 air changes per hour, maintained at 75 ± 5°F and 50 ± 10% relative humidity. The room air is passed through filters to remove 99.9% of all particles larger than 0.3 μ. Lighting is controlled by a timer providing 12 hr on and 12 hr off.

All animals were observed daily for toxic signs and behavioral changes and were provided medical treatment as necessary for nontest injuries under the supervision of our veterinary pathologists. The typical case was injuries due to fighting, which may be treated by isolation, cleaning the wounds, and antibiotic therapy, systemic and local.

B. Basic Protocol

1. Dose Levels and Treatment

We used a control group and three treatment groups, spaced at equal logarithmic intervals. With dogs, the doses of TNG in the respective treatment groups were 1 mg/kg/day (low), 5 mg/kg/day (middle), and 25 mg/kg/day (high). With the rodents, dosage levels of 0.01% (100 ppm), 0.1% (1000 ppm), and 1% (10,000 ppm) in the diet were used, respectively.

Dogs were dosed daily with capsules. Because of the bulk of the dosing mixture, high-dose dogs received two capsules of the 10% mixture. Middle-dose dogs received one capsule of the 10% mixture, and low-dose dogs received one capsule of a 2% mixture prepared by dilution with lactose in a ball mill. Control dogs received two capsules containing lactose. After each weekly weighing, capsules were prepared for each dog for the following week.

Rodent diets were prepared weekly. The 10% concentrate was mixed with feed in a rotating box on a modified cement mixer to provide the diet mixtures by successive dilutions. The control rodents received a mixture containing 10% dried feed in ordinary feed.

2. Number of Animals and Identification

Each group consisted of equal numbers of males and females. The beginning number of dogs was six of each sex per group, of rats 38 and of mice 58. Additional rats were included for the three-generation (20 females in each dosage group) and metabolism (12 males and 12 females, each, in the control, low dosage and high dosage group) studies. A few extra rodents were added to replace early losses.

Each animal is assigned a three- to five-digit number. The first two digits indicate the dosage groups for TNG, i.e., 80, 81, 82 and 83 for the control, low, middle or high dose group, respectively. The last one or two (dogs) or three (rodents) digits are the animal numbers within each species.

3. Schedule

a. Dogs

All the dogs were bled from their jugular veins for hematology and clinical chemistry tests before dosing and at the end of 3, 6, 9, and 12 months during dosing. They were weighed weekly. Feed consumption was measured 1 week each month. We originally intended to conduct a 24-month study. Near the end of the first year, observed effects were minimal. Our Advisory Committee concluded that we would learn little from

the second year of the study because only a lifetime (7-10 year) study could provide additional data. Therefore, after 12 months dosing, three male and three female dogs from each dosage group were killed for necropsy. The treatment of the remaining dogs in each group was discontinued for 4 weeks. These dogs were used on a recovery study and killed for necropsy at the end of 13 months.

b. Rats

Four males and four females from each dosage group were bled for hematology by cutting off their tail tips before dosing and at the end of 3, 6, 9, 12, 18 and 24 months during dosing. As much as possible, the same rats were used at each bleeding. If a bled rat died or his tail became too short, another rat was substituted. Rats were weighed weekly for the first 6 months; after weight gain leveled off, they were weighed biweekly. Feed consumption was measured during the first 4 weeks and then during the last week of each month. After 12 months dosing, four males and four females from each dosage group were bled from their aortas for clinical chemistry and killed for necropsy. A second group of four male and four female rats from each dosage group was started on a recovery study without treatment for 4 weeks. These rats were terminated at 13 months. After 24 months dosing, a similar recovery study was started and the remaining surviving rats were killed for necropsy, with eight from each group bled for clinical chemistry. The recovery rats were terminated at the 25th month.

c. Mice

Mice were weighed weekly for the first 5 months; after their weight gain leveled off, they were weighed biweekly. Feed consumption was measured during the first 4 weeks and then for 1 week each month thereafter. After 12 months dosing, four males and four females from each dosage group were bled from their aortas for hematology and killed for necropsy. A second group of four male and four female mice from each dosage group was started on a recovery study. After 24 months dosing, a similar recovery study was started and the other surviving mice killed for necropsy, with eight mice from each dosage group bled for hematology. The recovery mice were terminated at the 13th or 25th month, respectively.

C. Test Compound

1. Sources

A commercial mixture of 10% on lactose (SDM No. 17, Atlas Chemical Division, ICI America Inc., Wilmington, DE) was used to dose the dogs. The rodents were dosed with a concentrate prepared at Picatinny Arsenal, Dover,

NJ, under the supervision of Mr. Louis Avrami. We shipped a quantity of our feed (Wayne Lab-blox®, Allied Mills, Inc., Chicago, IL) to Picatinny. The feed was dried to 0.1% moisture, mixed with pure TNG to give a 10% mixture, packaged in plastic bags inside cardboard boxes and shipped to our underground magazine (Beyer Crushed Rock Co., Kansas City, MO). There we sampled the feed as it was transferred to 5-gal. glass jars, with aluminum foil sealed wooden plug tops held in place by springs. Exact concentration of TNG was determined to adjust the first dilution ratio to produce the desired concentration. Picatinny also shipped a quantity of the dried, TNG-free feed. This was added to control feed to correct for potential effects of the drying operation.

2. TNG Reference Sample Assay

Assay of a reference sample of TNG is discussed in Appendix III.

3. Extraction Procedure

A 2-g sample was transferred to a 30 ml bottle fitted with polyethylene seal cap. The sample was treated with 20 ml heptane and shaken for 20 min using a Burrell® wrist action shaker. A 5 ml aliquot was transferred to a 15 ml centrifuge tube and centrifuged for 10 min. A 1 ml aliquot was withdrawn and diluted with heptane such that the resulting solution concentration was about 5 ng TNG/ml.

4. Assay Procedure

Instrument: Bendix 2500 with ^{63}Ni electron capture detector.
Column: 1.83 x 2 mm I.D., glass
1.5% DC LSX-3-0295
1.5% GE x E-60 or Gas Chrom Q
Flow rate: 40 cc N_2/min
Temperatures: Column 130°C
Injector 135°C
Detector 200°C
Results: TNG elutes at 7 min. Use of a higher injection temperature causes decomposition.

5. Stability of Nitroglycerin on Rat Feed

Two feed levels of NG were prepared (0.5 and 0.05%). Duplicate samples were immediately taken and analyzed (Sample 1). Duplicate samples were also taken and frozen (Sample 2).

Two rat cage feeders per NG feed level were filled and stored in the normal manner. Duplicate samples were taken after 4 days from the feeders and frozen (Sample 3). After taking the 4 day samples the feeder contents were discarded.

Duplicate samples at each feed level were then taken from the original storage cans (Sample 4) and frozen. Part of these latter samples were placed in feeders in the normal manner then sampled after 4 days (Sample 5) and frozen. Duplicate samples were removed from the storage cans (Sample 6) and frozen. The results were:

Level about 0.5%

Sample	% NG	% Remaining
1. Time zero-fresh	0.41 ± 0.02	100 ± 5
2. Time zero-frozen plus 8 days	0.41 ± 0.02	100 ± 5
3. 4 days in feeder	0.39 ± 0.02	95 ± 5
4. Sample from can after 4 days storage	0.41 ± 0.03	100 ± 7
5. Sample No. 4 after 4 days in feeder	0.36 ± 0.02	88 ± 5
6. Sample from can after 8 days	0.38 ± 0.02	93 ± 5

Level about 0.05%

Sample	% NG	% Remaining
1. Time zero-fresh	0.042 ± 0.002	100 ± 5
2. Time zero-frozen plus 8 days	0.042 ± 0.002	100 ± 5
3. 4 days in feeder	0.040 ± 0.002	95 ± 5
4. Sample from can after 4 days storage	0.038 ± 0.002	90 ± 5
5. Sample No. 4 after 4 days in feeder	0.031 ± 0.004	74 ± 10

6. Secondary Amine Contamination

a. Rationale

When the presence of hepatic carcinogenesis was noted during the later portion of the study, exogenous carcinogens were considered. If aflatoxin or similar compounds were found in the feed, effects would be seen in all dosage groups. However, the TNG could react with endogenous compounds to form nitrosamines, whose concentration would be proportional to the TNG content. These hypothetical reactions would occur primarily in the 10% concentrate, since the dilutions are prepared, fed and discarded within 10 days.

The only nitrosatable compounds would be secondary amines. The only major natural secondary amines are proline and hydroxyproline. Although present in corn, fish, and other feedstuffs, essentially all is tied up in proteins, so there are negligible secondary amine groups available for reaction. Other natural secondary amines (spermine, spermidine, etc.) are trace constituents, if present at all, in feedstuffs. Diphenylamine is commonly used at concentrations of 1 or 2% in TNG and TNG mixtures (such as smokeless powders) as a stablizer. No diphenylamine should be in the TNG used here, but mistakes can occur. If it was added, some would probably react to form nitrosodiphenylamine, a known carcinogen.[28] Therefore we assayed the 10% TNG on feed concentrate for diphenylamine.

b. Methods

Samples of feed (control and 10% TNG) were extracted twice with 10 volumes of acetonitrile. Aliquots of the extract were analyzed by HPLC under these conditions:

Column: μ Bondapak C_{18}, 300 x 4 mm I.D.
Eluent: 60/40 acetonitrile/0.01M ammonium acetate, pH 4.0
Flow rate: 1 ml/min
Detector: UV, 286 nm
Injection: 15 to 20 μl

The amount present was quantified by the use of a standard addition of diphenylamine to duplicate samples.

c. Results

The method gave linear results over a concentration range of 100 (21 to 2,100 ng diphenylamine injected). No diphenylamine was found in control feed or in 10% TNG on feed. The limit of detection was 5 ng, so the concentration is less than 5 ppb on feed, or less than 50 ppb relative to TNG.

D. Procedures

1. Observation

All animals were observed daily for toxic signs and changes in behavior and general health.

2. Body Weights

Body weights were taken as mentioned above. Dogs were weighed to 0.1 kg, rodents to 1 g.

3. Measurement of Feed Consumption

The feed consumption of the dogs was measured by placing them in a metabolism cage, giving them a measured amount of feed, waiting 0.5 hr, then returning them to their pen and estimating the remaining amount of feed by volume. This value was converted to weight by a factor determined by averaging the weight of 20 replicates of volume measurements. Feed consumption of the rodents was determined by weighing the feed and container placed in the cage and that remaining 1 week later.

4. Unscheduled Deaths

If an animal appeared moribund, he was killed and necropsied as described below. If an animal was found dead, he was necropsied as thoroughly as possible, but no blood samples or organ weights were taken. If an animal received a serious injury or lesion, causing pain and suffering (such as an ulcerated tumor), he was killed and necropsied as if moribund.

E. Hematology and Clinical Chemistry

1. Hematology

The hematology battery included erythrocyte, reticulocyte, leucocyte and platelet counts, hematocrit, hemoglobin, erythrocyte indices, methemoglobin, Heinz bodies and (for dogs) clotting time. Details of methodology are summarized in Appendix I.

2. Clinical Chemistry

The clinical chemistry battery included fasting blood glucose, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, and blood urea nitrogen. Details of methodology appear in Appendix I.

3. Immunoglobin E

Immunoglobin E (IgE), the allergic or hypersensitive antibody, was associated with anaphylactic reactions in humans.[7] Serum concentrations

of IgE were determined in all clinical chemistry samples, using the immunodiffusion technique of Mancini.[8]

4. Special Tests

If indicated by symptoms or other test results, special tests such as serum electrolytes were performed.

5. Statistics

Data were analyzed using Dunnett's multiple comparison procedure following an analysis of variance, as described in Appendix I.

F. Necropsies

1. Killing and Gross Examination

Rodents were killed with ether and dogs with an overdose of sodium pentobarbital. The necropsy was performed as soon after death as possible. Gross abnormalities in all tissues are observed and recorded.

2. Organ Weights

The brain, heart, liver, kidneys, spleen, gonads, and (dogs only) adrenals, thyroids and pituitary were trimmed free from surrounding tissues and weighed. The absolute weights and organ weight to body weight and/or brain weight ratios were analyzed statistically. Abnormal growths were measured and, if practical, trimmed and weighed.

3. Histopathology

Tissues routinely taken for histopathologic examination are listed in Table 1. In addition, all tissues with gross abnormalities were taken. Processing is detailed in Appendix I.

G. Recovery Studies

Recovery studies were performed after each scheduled necropsy (12 and 24 months). The compound treatment of one male and one female dog or four rodents of each sex was discontinued. They were given the control treatment (lactose capsules or feed without compound, as appropriate) for 28 days. During this period, their weight and feed consumption were determined weekly. At termination, blood samples were taken from the jugular vein of dogs or the aorta of rodents for hematology and (except mice) clinical chemistry. The animals were then killed and necropsied. Detailed procedures are as given above.

H. Three-Generation Reproduction Study

1. Study Design

The study design is illustrated in Figure 1. The initial groups of rats used as the parental generation (F_0) were started at the same time as the chronic toxicity study. Rats of each group, parents and offspring of each generation, received the same control of TNG-containing diets as in the chronic study. For the F_0 generation, 10 males and 20 females from each dosage group were mated after receiving the test diets for 6 months. Each male was housed with two females from the same dosage group for 14 days. Offspring from the matings (F_{1a}, first litters) were discarded at weaning. The F_0 rats were again mated. Twenty to 24 offspring of each sex from this mating (F_{1b}, second litters) were randomly selected (with approximately equal numbers of pups from the various litters) from each dosage group at weaning. The F_0 females and surplus pups were discarded; the F_0 males were retained in the chronic study. Each F_{1b} male was mated with a female within the same dosage group for 14 days at 3 months of age. The F_{2a} generation was discarded at weaning and the F_{1b} rats were terminated at weaning of the F_{2b} pups. The F_{2b} rats were then selected and mated at 3 months of age according to the same procedure used for F_{1b}. The study was terminated upon weaning of the F_{3b} rats.

2. Evaluation

At birth, all offspring were examined for gross physical abnormalities and the number of live and dead pups of each litter were recorded. Survival and body weight were recorded at 0, 4 and 21 days.

Reproductive performance for each parental generation was quantified by: the mating ratio (the number of copulations to the number of male-female pairings), and fertility ratios for each sex (the number of males or females with offspring to the number of that sex mated). Reproductive performance for each litter was quantified by: the litter size, the liveborn index (the percentage of the total number of pups liveborn), the weight of liveborn pups at birth, the viability index (the percentage of the young alive at day 4 surviving to weaning), the weight at weaning, and the sex ratio (the number of males to the total number of offspring). Details of procedures are in Appendix II.

The general health of the parental generation was quantified by the weight at first mating.

I. Mutagenesis Studies

To assess the mutagenic potential of TNG, we performed cytogenetic analysis of tissue cultures from dogs and rats from the chronic toxicity study and dominant lethal mutation study in rats.

1. Cytogenetic Studies

a. Preparation of Cell Cultures

At the end of 1 year, blood samples were aseptically drawn from both control and treated dogs and rats. Blood was obtained from the tail vein of the rats and from the dogs' jugular veins. The lymphocytes were cultured by the method of Moorhead et al.[9] Bone marrow cells replaced peripheral blood lymphocytes as a source of mitotic chromosomes in the 2-year study. The use of bone marrow cells rather than peripheral blood lymphocytes has several advantages. Chromosomes will be obtained not only from lymphoidal cells but also from cells of myeloid, erythroid, and reticuloendotheloid origin. Another advantage of bone marrow cells is that the culture time is reduced from 72 hr needed in lymphocyte cultures to 24 hr and to mitogenic agent is required to obtain metaphase chromosomes. Femur bone marrow was removed at necropsy and processed by the method of Eggen;[10] bone marrow cultures were maintained in nutrient mixture F-12 (HAM). Kidney tissue samples were removed at necropsy, cultured by the trypsinization method of Fernandes,[11] and maintained in Eagle's medium as modified by Dulbecco and Vogt.[12]

b. Chromosome Analysis

Actively dividing kidney cultures, bone marrow cells, and phytohemaglutin-stimulated lymphocytes were arrested in metaphase by short-term colchicine treatment. The cells were removed from the culture flasks, swollen in hypotonic solution, and processed for spreading on glass slides by the method of Moorhead and Newell.[13] Slides were stained with Giemsa and scanned under low power optics. The slides showing minimum scattering of cells were selected for analysis under oil immersion optics. Cell ploidy was estimated by examination of 200 cells. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

2. Dominant Lethal Mutation Studies

Groups of male Charles River CD® rats were fed TNG in feed at the same dosage levels as the main toxicity study (0, 0.01, 0.1 and 1.0% TNG in feed) for 13 weeks, as specified below. Each male was then mated to two virgin females of the same strain. At mid-term of pregnancy, the females were killed and the following data collected: number of fertile males per number of males treated, number of pregnant females per number of mated females, number of corpora lutea per pregnant female, and the number of total implants, dead implants, and live implants per pregnant female. Methodology details are in Appendix II. Conclusive evidence for dominant lethality requires postimplantation losses. Increased preimplantation losses may be due to genetic damage.

J. Metabolism Studies

1. Experimental Procedure

Rats were fasted overnight for about 16 hr and given a single oral dose of approximately 1/10 of the LD50 of TNG. Males received a dose of 82 mg/kg and females a dose of 88 mg/kg. The compound, spiked with 25 μCi/kg of TNG-(1,3-^{14}C, specific activity of 53.25 mCi/mM), was suspended in peanut oil and given via an intragastric tube in a volume of 10 ml/kg of body weight. Immediately after dosing, each rat was placed in a Roth-Delmar metabolic cage for the separate collection of urine, feces and CO_2 in the expired air. The chamber was vented continuously with CO_2-free air at a rate of 250 ml/min. Expired CO_2 was collected by bubbling the air through six absorption columns connected in series. Each column contained 100 ml of 5% sodium hydroxide. Rats were given feed and water ad libitum. At the end of 24 hr, the rats were anesthetized with ether and blood was collected from the abdominal aorta. Various tissues were removed, weighed and processed for analysis of radioactivity.

2. Sample Preparation and Analysis

Volumes of urine and urine rinse were measured. Feces and GI tract (plus contents) were weighed and homogenized separately in 10 volumes of 80% methanol in a Waring blender. Whole blood (200-400 μl), fecal and GI homogenates (250-500 μl) and tissue samples (30-120 mg) were digested in 0.2 ml of 70% perchloric acid and 0.4 ml of hydrogen peroxide with heating at 75 to 80°C for $\simeq$ 4 hr. Ten ml of a toluene-PPO-dimethyl-POPOP cocktail containing 10% Beckman Biosolv BBS-3 were added to the digests or urine aliquot (100-200 μl). $^{14}CO_2$ samples from the air traps were spotted on filter paper, dried and counted. Samples were counted in duplicate in a Packard Tricarb (Model 3375) liquid scintillation counter. The counts were corrected for background and the counting efficiency was determined from a calibration curve obtained from a ^{14}C standard quench set (Amersham/Searle Corporation) using the external standard method.

3. Thin-Layer Chromatography (TLC) for Identification of Metabolites

Pre-coated silica gel plates (E. M. Laboratories, Inc., Elmsford, NY) having 0.25 mm thickness were used. Samples of raw urine or urine extracts were spotted $\simeq$ 2.0 cm from the bottom of the plate and developed for a minimum of 10 cm. Solvent systems used were: (a) benzene:ethylacetate (4:1, v/v); (b) ethylacetate:n-heptane (9:1, v/v); (c) n-butanol:acetate acid: water (5:1:4, v/v/v); and (d) n-butanol:methanol:water (120:33:57, v/v/v). A sample of pure TNG and reference standards available (dinitroglycerins, mononitroglycerins, glycerin) were spotted on each plate for reference. Nitroglycerins were detected using 5% diphenylamine spray reagent followed by UV-irradiation. Plates were air-dried and scraped into zones which were added to scintillation cocktail and counted directly. Metabolites remaining

at the origin after TLC development were scraped, eluted with water and treated with β-glucuronidase (Sigma Chemical Co.). The pH was adjusted to 5.6 by the addition of 13.6 mg/ml of sodium acetate. β-glucuronidase was added at a concentration of 5.0 mg/ml and the solution incubated at 37°C for 18 hr. After incubation, the solutions were extracted with 5 volumes of $CHCl_3$:MeOH (2:1). The resulting aqueous and organic phases were concentrated by evaporation and the metabolites were identified by TLC.

TABLE 1

ORGANS ROUTINELY TAKEN AT NECROPSY

Thyroid and parathyroids	Caecum
Pituitary	Colon
Adrenals	Urinary bladder
Lungs	Ureter[a/]
Liver and gallbladder	Diaphragm[a/]
Spleen	Skeletal muscle
Heart	Esophagus
Salivary glands	Tonsils[a/]
Pancreas	Mesenteric lymph node
Thymus	Tongue[a/]
Prescapular lymph node[a/]	Skin
Gonads	Mammary gland
Uterus or prostate and accessory organs	Brain
	Spinal cord[a/]
Stomach	Sciatic nerve[a/]
Duodenum	Eyes
Jejunum	Trachea
Ileum	Rib and bone marrow

a/ Not normally removed from rodents.

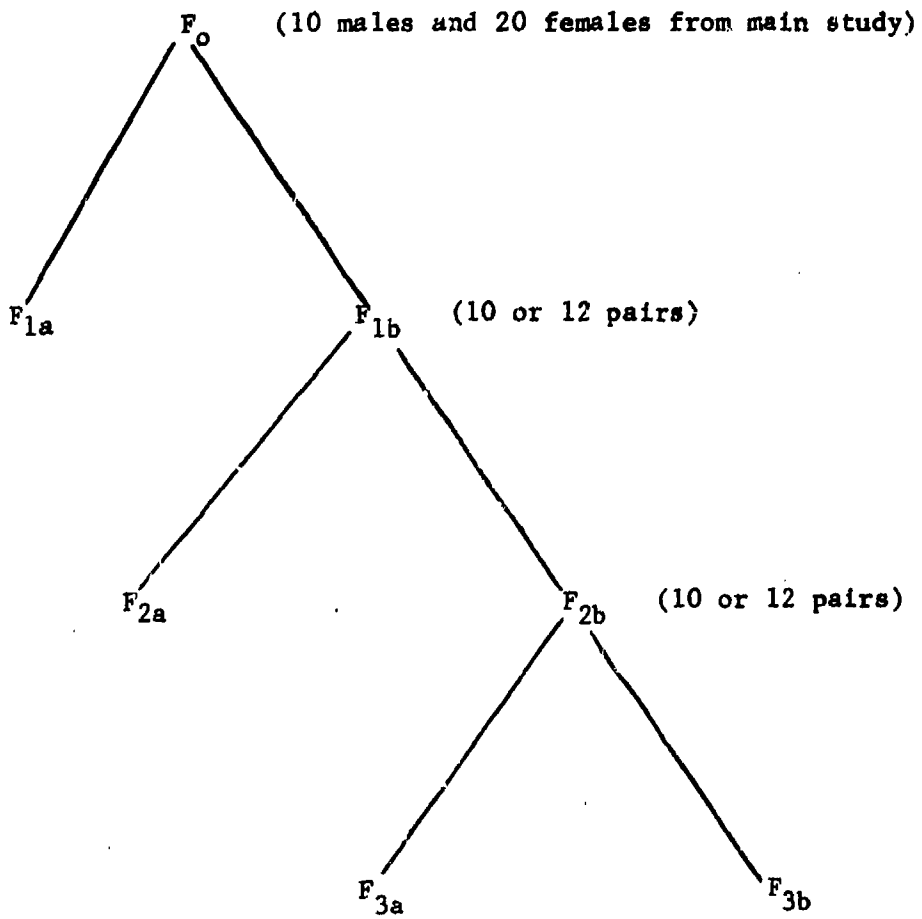

Figure 1 - Design of Three-Generation Reproduction Study

III. DOG STUDIES

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III. DOG STUDIES

The results and interpretation of the studies in dogs are described below.

A. Observations

The most significant observation in these dogs is that there were no toxic signs. The only incidents were those due to dogs scuffling in the runs. The most frequent victim was control male No. 80-1. As necessary, he was isolated while his wounds were treated with dressings and antibiotics. One morning on week 27 he was again found bleeding. The wounds appeared less bad than they had been on previous occasions. When we checked his dresings 90 min after they were applied, he was dead. Gross necropsy showed puncture wounds in the intestine, lungs and especially in the hind leg muscles. We ascribe his death to hemorrhagic shock from internal and external bleeding. An extra untreated male, purchased in the same group as the test dogs, was put on lactose capsules and used henceforth as a substitute No. 80-1.

B. Body Weight and Feed Consumption

The average body weights of dogs given various doses are shown in Figure 2. There are month-to-month variations, but no apparent dose effects. In males, the low-dose dogs were generally the heaviest, and the control dogs the lightest. In the females, body-weight averages were so close that the low- and middle-dose dogs have been omitted from the graph for clarity.

Average daily feed consumption is shown in Figure 3. Variation from month to month is extremely high, but there are no toxicologically important differences between the groups.

C. Laboratory Data

Baseline values of hematology and clinical chemistry for male and female dogs are shown in Tables 2 and 3, respectively. The subsequent tables show the values for these dogs after being treated with various doses of TNG for 3 months (Tables 4 and 5), 6 months (Tables 6 and 7), 9 months (Tables 8 and 9), 12 months (Tables 10 and 11) and 12 months followed by 1 month of recovery (Tables 12 and 13).

Before the start of the study, there were a few scattered differences between the dosage groups, but these are small and within normal limits (see Appendix I). After dosing for various periods, similar inconsistent differences were found.

The only consistent, toxicologically important finding was small amounts of methemoglobin in some dogs treated for 6 months or longer. In almost all cases, the amount found was very small (less than 3%). Because the assay method involves a difference in absorption values (before and after conversion of methemoglobin to cyanmethemoglobin; see Appendix I), a small apparent value could be an artifact. Therefore, we must draw inferences from the incidence, shown in Table 14. After 6 months, there was a scattered incidence in all groups; this is not significant. But after 9 months, there was methemoglobin in half or more of the low-dose males (given 1 mg/kg/day) and middle-dose (5 mg/kg/day) males and females, and all but one of the high-dose (25 mg/kg/day) dogs. The trend is significant in both sexes, although only the data in males are statistically significant. After 12 months, the dose response in incidence was not apparent. However, the high-dose male (5.3%) and one of the high-dose females (3.7%) had the highest values of methemoglobin seen in the entire study.

Since the only effect observed in these dogs was this methemoglobinemia, the recovery study was abbreviated to include only the erythrocyte parameters (Tables 21 and 13). Only one high-dose male had a methemoglobin level of 2.9%. It was probably an artifact.

D. Pathology

The absolute and relative organ weights from dogs given TNG for 12 months are shown in Table 15. Statistically significant, but toxicologically not important, differences were seen in the pituitary weights of middle-dose males and the heart weights (relative to brain) of high-dose males.

Gross pathology, such as lung nodules, correlated well with the histopathologic lesions (Table 16). None of the lesions, whether common (mild hepatocytic vesiculation) or rare (severe lymphocytic thyroiditis in No. 83-37 or the mild endometritis in No. 82-26) correlated with TNG treatment.

Since treatment of TNG for 12 months did not cause any lesions, the recovery study was truncated to hematology only with the approval of the technical monitor.

E. Cytogenetics

The cytogenetics slides were prepared after the 12-month necropsy, but, by agreement with the technical monitor, reading was deferred. Because the life-time (24 month) rat data (see below) showed no mutagenetic damage, despite considerable somatic lesions, while the shorter dog exposure was linked to negligible somatic lesions, the dog slides were not read.

F. Discussion

TNG is known to produce methemoglobin,[14] but it is a transient phenomenon, tending to disappear within hours.[3] Since blood samples were taken 20 or more hours after dosing, it was somewhat surprising to find any methemoglobin at all. Apparently, the amount found in this study was well within the body's ability to cope. None of the sequelae (Heinz bodies, reticulocytosis, anemia[15]) were evident.

G. Conclusions

The only effect which can be ascribed to TNG was occasional methemoglobinemia at all doses. The incidence and amount were dose related, only after administration of TNG for 9 months. The incidence extended to the low-dose males.

TABLE 2

LABORATORY DATA OF MALE DOGS BEFORE ADMINISTRATION OF TNG

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.75 ± .18	5.96 ± .17	5.97 ± .20	5.91 ± .10
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.47 ± .09	.43 ± .07	.54 ± .09	.66 ± .13
HEMATOCRIT, VOL. %	42.3 ± 1.5	41.8 ± 1.0	40.5 ± .6	42.0 ± 1.0
HEMOGLOBIN, GM. %	14.0 ± .5	14.0 ± .3	13.8 ± .2	14.1 ± .3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	73.7 ± 1.3	70.2 ± 1.3	68.0 ± 1.3[a/]	71.0 ± .7
MCHB, MICRO MICROGMS.	24.4 ± .4	23.5 ± .6	23.1 ± .4	23.8 ± .2
MCHBC, GM %	33.2 ± .3	33.5 ± .4	34.0 ± .2	33.5 ± .2
PLATELETS ($X10^5$ /MM^3)	3.3 ± .3	3.4 ± .2	3.6 ± .3	3.8 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	13.5 ± 1.0	13.7 ± .7	13.3 ± 1.3	13.5 ± .5
NEUTROPHILS, %	71.0 ± 1.3	60.5 ± 4.8	67.5 ± 2.0	61.7 ± 3.4
LYMPHOCYTES, %	26.0 ± 1.4	35.8 ± 4.1	30.8 ± 2.0	32.8 ± 3.8
BANDS, %	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .9	2.5 ± 1.2	1.0 ± .4	4.2 ± 1.4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.3 ± .7	1.2 ± .5	.7 ± .2	1.3 ± .7
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.8 ± .4	6.9 ± .3	6.7 ± .4	5.8 ± .3[a/]
GLUCOSE (FASTING), MG %	95.7 ± 4.3	86.0 ± 5.0	96.0 ± 4.4	100.3 ± 3.0
SGOT, IU/L	19.7 ± 1.8	21.2 ± 1.7	22.7 ± 2.3	27.0 ± 1.4[a/]
SGPT, IU/L	33.3 ± 3.0	37.5 ± 4.1	35.3 ± 2.9	33.8 ± 3.6
ALK. PHOS., IU/L	73 ± 9	75 ± 8	78 ± 6	69 ± 7
BUN, MG %	13.0 ± 1.9	13.7 ± .9	12.7 ± .8	12.7 ± .8
IMMUNOGLOBULIN E, IU/ML	1620 ± 75 (5)			1208 ± 58[a/]

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 3

LABORATORY DATA OF FEMALE DOGS BEFORE ADMINISTRATION OF TNG

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.76 ± .13	5.94 ± .15	6.28 ± .17[a/]	5.92 ± .12
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.37 ± .09	.48 ± .06	.58 ± .11	.68 ± .05[a/]
HEMATOCRIT, VOL. %	42.5 ± 1.0	41.5 ± .6	42.3 ± 1.0	41.7 ± .7
HEMOGLOBIN, GM. %	14.2 ± .2	13.9 ± .3	14.5 ± .4	13.9 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	73.8 ± 1.0	70.0 ± 1.5	67.5 ± .7[a/]	70.4 ± 1.2
MCHB, MICRO MICROGMS.	24.8 ± .3	23.5 ± .5	23.3 ± .3[a/]	23.4 ± .3[a/]
MCHBC, GM %	33.6 ± .3	33.6 ± .4	34.5 ± .3	33.3 ± .2
PLATELETS ($X10^5$ /MM^3)	3.4 ± .2	3.9 ± .3	3.5 ± .3	4.1 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	12.5 ± .8	13.6 ± .9	14.0 ± .5	12.4 ± .6
NEUTROPHILS, %	67.2 ± 2.2	64.2 ± 2.0	61.3 ± 2.1	58.8 ± 3.7
LYMPHOCYTES, %	29.7 ± 2.2	33.0 ± 1.7	36.5 ± 2.2	37.8 ± 4.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.5 ± 1.1	1.8 ± .6	1.7 ± .9	2.2 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.7 ± .2	1.0 ± .4	.5 ± .2	1.2 ± .4
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.9 ± .3	6.4 ± .4	6.3 ± .4	6.1 ± .1
GLUCOSE (FASTING), MG %	96.7 ± 2.6	95.0 ± 2.3	101.0 ± 2.1	98.8 ± 1.1
SGOT, IU/L	42.3 ± 22.0	23.8 ± 2.3	20.5 ± .9	23.7 ± 3.0
SGPT, IU/L	41.7 ± 10.6	58.7 ± 26.2	37.8 ± 4.4	34.8 ± 3.6
ALK. PHOS., IU/L	61 ± 2	69 ± 8	66 ± 6	62 ± 5
BUN, MG %	14.5 ± 1.2	14.3 ± .8	13.7 ± .9	11.8 ± 1.1
IMMUNOGLOBULIN E, IU/ML	1454 ± 86			1342 ± 53

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 4

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF TMG FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.88 ± .20	5.88 ± .15	5.86 ± .15	5.89 ± .15
HEINZ BODIES. %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES. %	.70 ± .05	.76 ± .13	.58 ± .07	.69 ± .12
HEMATOCRIT. VOL. %	42.2 ± .9	41.2 ± 1.2	41.0 ± 1.0	41.5 ± 1.2
HEMOGLOBIN. GM. %	14.3 ± .4	14.2 ± .4	14.1 ± .3	14.4 ± .4
METHEMOGLOBIN. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV. CUBIC MICRONS	71.9 ± 1.3	70.1 ± 1.2	70.0 ± 1.1	70.5 ± .6
MCHB. MICRO MICROGMS.	24.4 ± .4	24.1 ± .3	24.2 ± .4	24.5 ± .2
MCHBC. GM %	34.0 ± .5	34.5 ± .1	34.5 ± .1	34.8 ± .4
PLATELETS ($X10^5$ /MM^3)	2.7 ± .1	2.8 ± .3	2.8 ± .1	2.9 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	12.8 ± 1.0	12.3 ± .9	11.0 ± .6	11.0 ± .9
NEUTROPHILS. %	65.5 ± 3.4	57.7 ± 2.1	61.2 ± 2.9	58.7 ± 2.9
LYMPHOCYTES. %	28.8 ± 3.8	36.8 ± 2.4	31.8 ± 2.1	35.3 ± 3.6
BANDS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS. %	4.8 ± 1.1	5.2 ± 1.2	6.7 ± 1.1	5.7 ± 1.9
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	.8 ± .3	.3 ± .2	.3 ± .2	.3 ± .2
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME. MIN.	8.5 ± .2	8.3 ± .4	8.8 ± .4	8.8 ± .5
GLUCOSE (FASTING). MG %	90.0 ± 2.6	88.0 ± 2.8	91.5 ± 2.7	90.3 ± 2.9
SGOT. IU/L	32.5 ± 1.0	33.2 ± 2.9	31.3 ± 1.6	47.3 ± 12.9
SGPT. IU/L	35.8 ± 2.9	45.8 ± 10.8	38.5 ± 2.9	40.3 ± 7.2
ALK. PHOS.. IU/L	49 ± 6	53 ± 5	57 ± 7	47 ± 5
BUN. MG %	13.0 ± .8	12.5 ± .8	13.0 ± .6	13.2 ± .9
IMMUNOGLOBULIN E. IU/ML	2021 ± 183			2150 ± 123

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 5

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF TMC FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	6.13 ± .14	5.86 ± .14	6.24 ± .25	6.18 ± .14
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.52 ± .10	.51 ± .05	.69 ± .16	.82 ± .13
HEMATOCRIT, VOL. %	43.0 ± .5	41.8 ± .7	44.2 ± 1.1	43.8 ± 1.0
HEMOGLOBIN, GM. %	14.4 ± .2	14.3 ± .3	15.2 ± .4	15.0 ± .3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	70.3 ± 1.4	71.4 ± 1.2	71.1 ± 1.5	70.9 ± 1.2
MCHb, MICRO MICROGMS.	24.3 ± .4	24.4 ± .4	24.4 ± .4	24.3 ± .4
MCHbC, GM %	34.6 ± .4	34.2 ± .4	34.4 ± .2	34.2 ± .2
PLATELETS ($X10^5$ /MM^3)	2.8 ± .2	2.6 ± .4	3.0 ± .3	2.7 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	11.1 ± .8	11.9 ± 1.7	12.2 ± .6	12.2 ± 1.0
NEUTROPHILS, %	55.3 ± 2.0	64.7 ± 2.8	55.8 ± 3.4	58.2 ± 3.1
LYMPHOCYTES, %	37.7 ± 2.5	31.2 ± 2.6	39.3 ± 3.2	38.0 ± 3.9
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	5.8 ± .9	3.8 ± .7	4.5 ± .6	3.5 ± 1.1
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.2 ± .5	.3 ± .2	.3 ± .3	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.4 ± .4	7.5 ± .3	7.3 ± .3	7.2 ± .4
GLUCOSE (FASTING), MG %	92.0 ± 1.0	96.3 ± 3.0	97.0 ± 3.0	94.0 ± 3.6
SGOT, IU/L	33.0 ± 1.5	35.3 ± 3.8	30.8 ± 2.3	33.0 ± 1.0
SGPT, IU/L	33.5 ± 2.4	43.2 ± 9.7	39.0 ± 2.0	35.5 ± 1.5
ALK. PHOS., IU/L	41 ± 2	52 ± 7	48 ± 5	45 ± 6
BUN, MG %	12.2 ± .3	12.2 ± .3	14.3 ± .9	13.3 ± .8
IMMUNOGLOBULIN E, IU/ML	2083 ± 139			2333 ± 96

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 6

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF TNG FOR 6 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	6.07 ± .21	6.13 ± .15	5.86 ± .26	5.81 ± .20
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.77 ± .16	.69 ± .06	.80 ± .09	.91 ± .06
HEMATOCRIT, VOL. %	44.7 ± 1.1	44.0 ± 1.0	42.7 ± 1.3	43.5 ± 1.6
HEMOGLOBIN, GM. %	14.8 ± .3	14.8 ± .4	14.4 ± .4	14.7 ± .5
METHEMOGLOBIN, %	1.1 ± .2	.5 ± .3	.9 ± .3	1.5 ± .2
MCV, CUBIC MICRONS	73.8 ± 1.2	71.8 ± .9	73.0 ± 1.2	74.9 ± 1.4
MCHA, MICRO MICROGMS.	24.5 ± .3	24.1 ± .3	24.6 ± .4	25.4 ± .6
MCHHC, GM %	33.2 ± .2	33.6 ± .2	33.7 ± .1	33.9 ± .3
PLATELETS ($X10^5$ /MM^3)	3.1 ± .1	3.2 ± .2	3.2 ± .2	3.4 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	13.0 ± .4	13.9 ± 1.3	13.3 ± 1.0	13.4 ± 1.3
NEUTROPHILS, %	67.7 ± 2.9	62.7 ± 2.2	67.3 ± 3.5	62.8 ± 3.5
LYMPHOCYTES, %	29.5 ± 3.1	29.7 ± 2.0	28.0 ± 2.4	31.8 ± 2.9
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.8 ± .5	6.8 ± .7[a/]	4.2 ± 1.6	4.7 ± 1.1
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.8 ± .4	.5 ± .5	.7 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	9.2 ± .3	8.2 ± .4	8.8 ± .5	8.6 ± .2
GLUCOSE (FASTING), MG %	80.2 ± 2.0	83.7 ± 1.7	85.2 ± 1.2	83.5 ± 1.9
SGOT, IU/L	28.7 ± 1.7	25.0 ± 2.8	23.2 ± 1.1	29.3 ± 1.8
SGPT, IU/L	40.2 ± 3.9	36.0 ± 2.9	35.0 ± 3.7	34.3 ± 2.5
ALK. PHOS., IU/L	33 ± 4	33 ± 4	48 ± 8	34 ± 5
BUN, MG %	15.3 ± 1.6	15.0 ± .8	15.2 ± .6	13.2 ± .9
IMMUNOGLOBULIN E, IU/ML	433 ± 54			492 ± 100

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 7

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF THG FOR 6 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.94 ± .22	5.94 ± .21	5.70 ± .14	5.78 ± .16
HEINZ BODIES, %	0.00 ± 0.00	.05 ± .04	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.84 ± .18	.49 ± .14	.88 ± .03	1.11 ± .21
HEMATOCRIT, VOL, %	43.2 ± 1.1	43.3 ± .9	42.0 ± 1.3	44.8 ± 1.4
HEMOGLOBIN, GM, %	14.8 ± .6	14.8 ± .5	14.2 ± .4	14.3 ± .5
METHEMOGLOBIN, %	.3 ± .3	0.0 ± 0.0	.2 ± .2	.2 ± .2
MCV, CUBIC MICRONS	73.0 ± 2.3	73.2 ± 1.8	73.7 ± 2.0	77.8 ± 2.5
MCHB, MICRO MICROGMS.	24.9 ± .3	25.0 ± .3	24.9 ± .4	24.8 ± .6
MCHBC, GM %	34.3 ± 1.1	34.2 ± .6	33.9 ± .9	32.0 ± .6
PLATELETS ($X10^5$ /MM^3)	3.4 ± .2	3.3 ± .3	3.0 ± .3	3.3 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	12.2 ± .9	12.0 ± .9	13.0 ± .4	13.0 ± .7
NEUTROPHILS, %	58.8 ± 3.3	55.3 ± 1.1	63.8 ± 3.3	66.8 ± 3.5
LYMPHOCYTES, %	38.8 ± 2.6	41.2 ± 2.5	30.8 ± 3.6	28.3 ± 4.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.3 ± .9	3.2 ± 1.6	4.8 ± 1.5	4.7 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.3 ± .2	.5 ± .2	.2 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.8 ± .4	7.8 ± .3	8.3 ± .4	8.2 ± .3
GLUCOSE (FASTING), MG %	90.5 ± 1.6	95.8 ± 2.3	98.8 ± 2.3[a/]	96.0 ± 1.2
SGOT, IU/L	32.2 ± 6.4	28.5 ± 2.3	27.5 ± 3.4	24.8 ± 2.2
SGPT, IU/L	40.8 ± 7.4	42.2 ± 5.8	38.5 ± 3.2	38.0 ± 5.8
ALK. PHOS., IU/L	25 ± 3	34 ± 5	41 ± 4[a/]	37 ± 5
BUN, MG %	14.0 ± .9	16.3 ± 1.0	12.7 ± .6	14.0 ± .5
IMMUNOGLOBULIN E, IU/ML	517 ± 92			492 ± 85

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 8

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF TNG FOR 9 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	6.36 ± .26	6.56 ± .12	6.56 ± .15	6.35 ± .11
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.51 ± .11	.57 ± .08	.58 ± .09	.55 ± .10
HEMATOCRIT, VOL. %	45.0 ± 1.4	45.3 ± 1.0	44.8 ± .4	44.8 ± .9
HEMOGLOBIN, GM. %	15.4 ± .5	15.4 ± .3	15.5 ± .1	15.5 ± .3
METHEMOGLOBIN, %	0.0 ± 0.0	.9 ± .5	.9 ± .3	1.1 ± .2[a/]
MCV, CUBIC MICRONS	70.9 ± 1.0	69.1 ± .8	68.5 ± 1.3	70.6 ± .4
MCHB, MICRO MICROGMS.	24.3 ± .3	23.4 ± .2	23.7 ± .4	24.3 ± .2
MCHBC, GM %	34.2 ± .4	33.9 ± .3	34.6 ± .3	34.5 ± .2
PLATELETS ($X10^5$ /MM^3)	2.4 ± .1	2.6 ± .3	2.5 ± .1	2.6 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	13.3 ± 1.2	11.0 ± .9	10.4 ± 1.3	10.1 ± 1.5
NEUTROPHILS, %	72.0 ± 1.5	66.0 ± 2.9	65.5 ± 2.2	56.2 ± 3.7[a/]
LYMPHOCYTES, %	22.7 ± 2.1	26.3 ± 2.6	27.5 ± 2.1	33.7 ± 2.3[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.3 ± .6	6.3 ± 1.1	5.5 ± 1.3	9.2 ± 2.4[a/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.7 ± .7	1.3 ± .4	1.5 ± .6	1.0 ± .3
ATYPICAL, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME - MIN.	9.3 ± .7	8.8 ± .4	10.8 ± .8	12.2 ± .7[a/]
GLUCOSE (FASTING), MG %	70.5 ± 1.5	78.0 ± 2.5	79.7 ± 4.3	72.3 ± 1.6
SGOT, IU/L	36.0 ± 3.1	31.3 ± 2.9	37.5 ± 2.5	29.8 ± 2.0
SGPT, IU/L	38.5 ± 3.4	41.0 ± 4.3	46.8 ± 5.4	39.0 ± 3.5
ALK. PHOS., IU/L	31 ± 3	32 ± 4	45 ± 9	34 ± 6
BUN, MG %	13.0 ± 1.1	11.0 ± .9	12.7 ± .6	12.2 ± .3
IMMUNOGLOBULIN E, IU/ML	633 ± 122			542 ± 66

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 9

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF TNG FOR 9 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	1 (T. 6)	5 (T. 6)	25 (T. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	6.29 ± .14	5.99 ± .22	6.68 ± .22	6.16 ± .15
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.63 ± .19	.72 ± .15	.49 ± .09	.66 ± .09
HEMATOCRIT, VOL. %	45.0 ± .9	42.7 ± 1.1	46.0 ± 1.4	43.5 ± 1.9
HEMOGLOBIN, GM. %	15.3 ± .3	14.5 ± .6	16.2 ± .6	15.5 ± .5
METHEMOGLOBIN, %	.5 ± .5	0.0 ± 0.0	.9 ± .5	1.6 ± .2
MCV, CUBIC MICRONS	71.6 ± 1.1	71.4 ± .8	68.9 ± .6	70.4 ± 1.4
MCHH, MICRO MICROGMS.	24.4 ± .4	24.2 ± .2	24.3 ± .2	25.1 ± .5
MCHBC, GM %	34.1 ± .3	34.0 ± .5	35.3 ± .4	35.7 ± 1.2
PLATELETS ($X10^5$ /MM^3)	2.5 ± .2	2.9 ± .3	2.6 ± .2	3.3 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	10.6 ± .9	10.8 ± .7	10.3 ± 1.1	11.3 ± .9
NEUTROPHILS, %	64.8 ± 3.5	58.2 ± 2.0	61.0 ± 2.1	60.0 ± 3.1
LYMPHOCYTES, %	29.2 ± 3.0	34.3 ± 2.4	33.7 ± 2.3	32.8 ± 2.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2
EOSINOPHILS, %	4.3 ± 1.3	6.3 ± 1.5	4.2 ± .8	5.7 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.8 ± .5	1.2 ± .8	1.2 ± .5	1.3 ± .5
ATYPICAL, %	.5 ± .5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0
CLOTTING TIME, MIN.	10.7 ± .3	9.8 ± .8	10.8 ± .5	9.7 ± .4
GLUCOSE (FASTING), MG %	85.5 ± 2.5	75.8 ± 2.6	80.5 ± 3.6	74.8 ± 2.5 a/
SGOT, IU/L	34.5 ± 2.6	29.5 ± 4.0	37.8 ± 4.7	31.5 ± 1.4
SGPT, IU/L	38.0 ± 1.7	48.3 ± 20.8	33.8 ± 3.6	35.0 ± 3.2
ALK. PHOS., IU/L	31 ± 3	37 ± 8	34 ± 6	33 ± 5
BUN, MG %	12.0 ± .6	10.5 ± .6	13.3 ± 1.7	11.8 ± .7
IMMUNOGLOBULIN E, IU/ML	725 ± 275			567 ± 117

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 10

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF TNG FOR 12 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 6)	1 (T, 6)	5 (T, 6)	25 (T, 6)
ERYTHROCYTES ($X10^6$ /MM^3)	7.29 ± .21	7.13 ± .31	6.65 ± .14	6.53 ± .19
HEINZ BODIES, %	.01 ± .01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.42 ± .08	.44 ± .07	.65 ± .09	.63 ± .11
HEMATOCRIT, VOL. %	47.2 ± .9	46.7 ± 1.3	46.7 ± .7	46.7 ± 1.5
HEMOGLOBIN, GM. %	16.3 ± .3	16.2 ± .4	16.4 ± .2	16.4 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	.2 ± .2	.5 ± .5	.9 ± .9
MCV, CUBIC MICRONS	64.9 ± 2.2	65.7 ± 1.4	70.3 ± 1.4	71.5 ± .6[a/]
MCHB, MICRO MICROGMS.	22.5 ± .8	22.8 ± .7	24.7 ± .4[a/]	25.1 ± .4[a/]
MCHBC, GM %	34.6 ± .1	34.7 ± .4	33.1 ± .1	35.2 ± .3
PLATELETS ($X10^5$ /MM^3)	2.3 ± .1	2.7 ± .4	2.5 ± .2	2.8 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	11.1 ± .4	11.5 ± 1.1	12.4 ± 1.4	12.2 ± 1.0
NEUTROPHILS, %	70.8 ± 1.3	67.0 ± 1.9	68.3 ± 2.0	59.7 ± 3.0[a/]
LYMPHOCYTES, %	23.7 ± 1.3	26.2 ± 2.9	25.3 ± 2.4	32.2 ± 3.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	5.5 ± 1.1	6.8 ± 1.4	6.3 ± 1.4	8.2 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	10.2 ± .4	10.8 ± .4	9.8 ± .8	9.9 ± .6
GLUCOSE (FASTING), MG %	84.5 ± 2.3	79.8 ± 3.0	89.8 ± 2.9	87.7 ± 2.0
SGOT, IU/L	28.3 ± 1.3	33.3 ± 3.6	31.3 ± 1.9	31.5 ± 1.4
SGPT, IU/L	36.8 ± 3.5	41.5 ± 2.3	42.0 ± 3.3	41.0 ± 3.5
ALK. PHOS., IU/L	27 ± 3	31 ± 7	35 ± 7	27 ± 5
BUN, MG %	13.0 ± 1.3	13.8 ± .5	13.8 ± .8	14.7 ± 1.1
IMMUNOGLOBULIN E, IU/ML	400 ± 206			583 ± 53

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 11

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF TNG FOR 12 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 6)	1 (T, 6)	5 (T, 6)	25 (T, 6)
ERYTHROCYTES ($X10^6$ /MM^3)	7.08 ± .35	6.90 ± .21	6.55 ± .23	6.24 ± .18
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.50 ± .06	.48 ± .12	.74 ± .11	.59 ± .11
HEMATOCRIT, VOL. %	46.5 ± 1.3	46.7 ± 1.5	46.3 ± 1.5	45.3 ± 1.3
HEMOGLOBIN, GM. %	16.1 ± .6	16.3 ± .6	16.3 ± .5	15.8 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	.2 ± .2	.4 ± .2	.8 ± .6
MCV, CUBIC MICRONS	66.1 ± 1.8	67.7 ± 1.5	70.8 ± .6	72.7 ± 1.2[a/]
MCHB, MICRO MICROGMS.	22.9 ± .6	23.6 ± .5	24.9 ± .4[a/]	25.4 ± .5[a/]
MCHBC, GM %	34.7 ± .4	34.9 ± .3	35.2 ± .3	34.9 ± .3
PLATELETS ($X10^5$ /MM^3)	2.4 ± .3	2.6 ± .3	2.5 ± .2	3.1 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	10.4 ± .7	12.1 ± .8	11.8 ± .7	11.8 ± .4
NEUTROPHILS, %	67.0 ± 4.2	66.5 ± 3.7	64.7 ± 2.0	51.8 ± 9.7
LYMPHOCYTES, %	26.2 ± 2.3	25.8 ± 2.7	32.3 ± 2.6	39.2 ± 4.7[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	6.8 ± 3.4	7.7 ± 1.1	3.0 ± 1.5	8.7 ± 4.9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.6 ± .8	8.0 ± .9	7.3 ± .3	8.2 ± .5
GLUCOSE (FASTING), MG %	86.8 ± 3.4	90.8 ± 3.1	93.7 ± 1.1	93.7 ± 1.0
SGOT, IU/L	29.7 ± 3.1	24.2 ± 1.5	26.5 ± 1.2	29.7 ± 2.2
SGPT, IU/L	32.0 ± 2.1	34.8 ± 3.9	37.3 ± 3.6	35.5 ± 2.5
ALK. PHOS., IU/L	23 ± 2	33 ± 4	32 ± 3	32 ± 4
BUN, MG %	12.7 ± 1.2	14.0 ± .9	14.2 ± 1.2	14.7 ± .6
IMMUNOGLOBULIN E, IU/ML	667 ± 53			500 ± 0[a/]

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 12

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF TNG FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 3)	1 (T. 3)	5 (T. 3)	25 (T. 3)
ERYTHROCYTES ($X10^6$ /MM^3)	6.03 ± .20	6.17 ± .14	6.61 ± .18	6.72 ± .07[a/]
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.35 ± .07	.59 ± .11	.49 ± .14	.43 ± .13
HEMATOCRIT, VOL. %	45.0 ± 1.0	43.3 ± 1.3	46.7 ± 1.3	46.3 ± 1.9
HEMOGLOBIN, GM. %	15.4 ± .6	15.2 ± .4	15.1 ± .4	15.7 ± .8
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0
MCV, CUBIC MICRONS	74.7 ± 1.2	70.2 ± .7	70.6 ± 1.1	68.9 ± 2.8
MCHB, MICRO MICROGMS.	25.6 ± .5	24.6 ± .1	24.3 ± .1	23.3 ± 1.1
MCHBC, GM %	34.2 ± .5	35.0 ± .3	34.4 ± .3	33.8 ± .4

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 13

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF TNG FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 3)	1 (T, 3)	5 (T, 3)	25 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	6.38 ± .08	6.84 ± .43	6.52 ± .24	6.55 ± .37
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.65 ± .17	.59 ± .19	.62 ± .04	.52 ± .10
HEMATOCRIT, VOL, %	48.0 ± 1.0	48.3 ± 2.2	46.7 ± 2.9	49.7 ± 2.0
HEMOGLOBIN, GM, %	16.4 ± .4	17.0 ± .8	15.7 ± .9	16.3 ± .8
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	75.3 ± 1.6	70.9 ± 1.7	71.4 ± 1.9	76.0 ± 2.2
MCHB, MICRO MICROGMS.	25.7 ± .5	24.9 ± .6	24.0 ± .5	25.0 ± 1.2
MCHBC, GM %	34.2 ± .1	35.1 ± .1	33.6 ± .2	32.9 ± .7

ENTRIES ARE MEAN ± STANDARD ERROR.

a/ Significantly different from control dogs (Dunnett's multiple comparison procedure).

TABLE 14

INCIDENCE OF METHEMOGLOBIN IN DOGS GIVEN TNG

Treatment Period (months):	6		9		12		13[a]	
Sex:	M	F	M	F	M	F	M	F
Dose (mg/kg/day)								
0	5/6[b]	1/6	0/6	1/6	0/6	0/6	0/3	0/3
1	2/6	0/6	3/6	0/6	1/6	1/6	0/3	0/3
5	4/6	1/6	4/6	3/6	1/6	2/6	0/3	0/3
25	6/6	1/6	5/6	6/6	1/6	2/6	1/3	0/3

a/ Given TNG for 12 months and allowed to recover for 1 month.
b/ Dogs with methemoglobinemia/dogs tested.

TABLE 15

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS GIVEN TNG FOR 12 MONTHS

Sex	Dose (mg/kg/day)	Terminal Body Weight (kg)	Absolute Organ Weight (g)								
			Brain	Heart	Liver	Kidney	Spleen	Thyroid	Pituitary	Testis	Ovary
Male	0	12.1 ± 0.3[a]	80.3 ± 1.[illegible]	109.9 ± 1.5	372.3 ± 11.0	68.6 ± 4.7	80.6 ± 14.8	0.91 ± 0.08	0.08 ± 0.00	15.5 ± 0.5	
	1	11.8 [illegible]	82.4 ± 3.[illegible]	95.2 ± 7.7	327.9 ± 46.2	61.1 ± 4.2	80.8 ± 7.3	1.01 ± 0.13	0.07 ± 0.01	17.8 ± 1.8	
	5	10.7 ± [illegible]	80.3 ± 2.[illegible]	92.1 ± 3.5	302.9 ± 9.3	67.3 ± 7.7	58.0 ± 19.4	0.78 ± 0.11	0.06 ± 0.00[b]	17.6 ± 1.7	
	25	10.5 ± [illegible]	80.6 ± 1.6	89.1 ± 5.9	337.7 ± 10.0	64.2 ± 6.5	70.5 ± 4.3	1.02 ± 0.12	0.06 ± 0.00	15.2 ± 1.8	
Female	0	11.7 ± 1.3	86.1 ± 6.6	96.4 ± 12.4	335.3 ± 47.0	56.0 ± 5.5	87.2 ± 12.0	0.85 ± 0.20	0.07 ± 0.01		1.15 ± 0.39
	1	10.3 ± 0.7	81.4 ± 3.4	80.1 ± 5.9	285.6 ± 8.0	52.0 ± 3.1	69.5 ± 13.9	0.73 ± 0.04	0.07 ± 0.01		1.43 ± 0.10
	5	10.4 ± 0.6	80.7 ± 1.7	72.8 ± 5.6	352.7 ± 29.6	51.7 ± 6.6	63.4 ± 8.3	0.84 ± 0.06	0.08 ± 0.01		1.60 ± 0.09
	25	9.9 ± 1.0	80.0 ± 4.9	76.5 ± 8.2	328.8 ± 26.1	49.0 ± 1.5	66.1 ± 9.0	0.84 ± 0.05	0.07 ± 0.01		1.29 ± 0.29

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/kg body weight)								
		Brain	Heart	Liver	Kidney	Spleen	Thyroid	Pituitary	Testis	Ovary
Male	0	6.64 ± 0.03	9.10 ± 0.35	30.84 ± 1.53	5.68 ± 0.46	6.69 ± 1.29	0.068 ± 0.008	0.007 ± 0.000	1.28 ± 0.04	
	1	7.12 ± 0.68	8.17 ± 0.61	27.62 ± 1.49	5.23 ± 0.19	7.04 ± 1.11	0.086 ± 0.007	0.006 ± 0.000	1.51 ± 0.01	
	5	7.52 ± 0.09	8.63 ± 0.26	28.39 ± 0.46	6.32 ± 0.75	5.44 ± 1.84	0.074 ± 0.011	0.005 ± 0.000[b]	1.64 ± 0.15	
	25	7.69 ± 0.45	8.45 ± 0.19	32.10 ± 0.47	6.06 ± 0.35	6.69 ± 0.18	0.096 ± 0.008	0.006 ± 0.000	1.43 ± 0.10	
Female	0	7.45 ± 0.31	8.31 ± 0.77	28.70 ± 2.40	4.82 ± 0.09	7.47 ± 0.43	0.072 ± 0.011	0.006 ± 0.000		0.094 ± 0.022
	1	7.97 ± 0.37	7.80 ± 0.12	28.16 ± 2.72	5.07 ± 0.16	6.97 ± 1.72	0.071 ± 0.002	0.007 ± 0.001		0.139 ± 0.004
	5	7.79 ± 0.47	6.98 ± 0.41	33.78 ± 1.97	4.92 ± 0.38	6.04 ± 0.54	0.082 ± 0.010	0.007 ± 0.001		0.153 ± 0.003
	25	8.20 ± 0.52	7.76 ± 0.06	34.41 ± 5.86	5.09 ± 0.64	6.74 ± 0.77	0.088 ± 0.011	0.007 ± 0.001		0.138 ± 0.041

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/g brain weight)							
		Heart	Liver	Kidney	Spleen	Thyroid	Pituitary	Testis	Ovary
Male	0	1.37 ± 0.05	4.64 ± 0.21	0.86 ± 0.07	1.01 ± 0.19	0.010 ± 0.001	0.0010 ± 0.0000	0.19 ± 0.01	
	1	1.15 ± 0.05	4.00 ± 0.61	0.74 ± 0.05	0.99 ± 0.13	0.012 ± 0.001	0.0008 ± 0.0001	0.22 ± 0.02	
	5	1.15 ± 0.04	3.77 ± 0.02	0.84 ± 0.11	0.72 ± 0.24	0.010 ± 0.002	0.0007 ± 0.0000	0.22 ± 0.02	
	25	1.11 ± 0.09[b]	4.19 ± 0.20	0.80 ± 0.09	0.88 ± 0.06	0.013 ± 0.002	0.0008 ± 0.0001	0.19 ± 0.03	
Female	0	1.12 ± 0.11	3.87 ± 0.38	0.65 ± 0.02	1.01 ± 0.07	0.010 ± 0.001	0.0008 ± 0.0001		0.013 ± 0.004
	1	0.98 ± 0.04	3.52 ± 0.38	0.64 ± 0.01	0.87 ± 0.20	0.009 ± 0.000	0.0009 ± 0.0000		0.018 ± 0.001
	5	0.90 ± 0.06	4.37 ± 0.34	0.64 ± 0.08	0.79 ± 0.11	0.011 ± 0.001	0.0009 ± 0.0001		0.020 ± 0.001
	25	0.95 ± 0.05	4.14 ± 0.43	0.62 ± 0.04	0.82 ± 0.07	0.011 ± 0.001	0.0008 ± 0.0001		0.016 ± 0.004

a/ Mean ± standard error of three dogs.

b/ Significantly different from control by Dunnett's Multiple Comparison Procedure.

TABLE 16

SUMMARY OF LESIONS AND M/E RATIOS IN DOGS
GIVEN TNG FOR 12 MONTHS

Dose (mg/kg/day):	0						1						5						25					
Dog No.:	1	2	3	4	5	6	13	14	15	16	17	18	25	26	27	28	29	30	37	38	39	40	41	42
Sex:	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Lesions[a/]																								
Adrenal																								
Focal fatty change					1		1		1															
Thyroid																								
Chronic lymphocytic thyroiditis																			4					
Lung																								
Focal fibrosis and granuloma		1	1		1				1						1								1	1
Peribronchiolar cuffing							1		1	1													1	1
Liver																								
Portal inflammation							2	1																
Hepatocyte vesiculation	1	1	1	1	1	1			1			1	1	1	1	2		1	1		1	1	1	1
Cystic hyperplasia of gallbladder	1																		1					
Bile duct hyperplasia									1															
Focal necrosis										1														
Parasite migration scar							X																	
Salivary gland																								
Focal mononuclear cell infiltration	1	1			1	1		1													1			
Uterus																								
Endometritis														1										
Kidney																								
Microscopic calculi														1				1						
Focal mononuclear cells infiltration																	1							
Lymph Node																								
Eosinophilic granuloma			1														1		1				1	
Eye																								
Hyperplasia of glans nictatans			4																					
Bone Marrow																								
M/E ratio	1.0	0.8	1.2	1.2	1.0	1.3	1.3	0.8	1.3	1.8	1.1	1.0	1.8	1.6	1.1	0.7	2.3	1.0	1.8	1.1	0.8	0.8	1.0	1.1

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present.

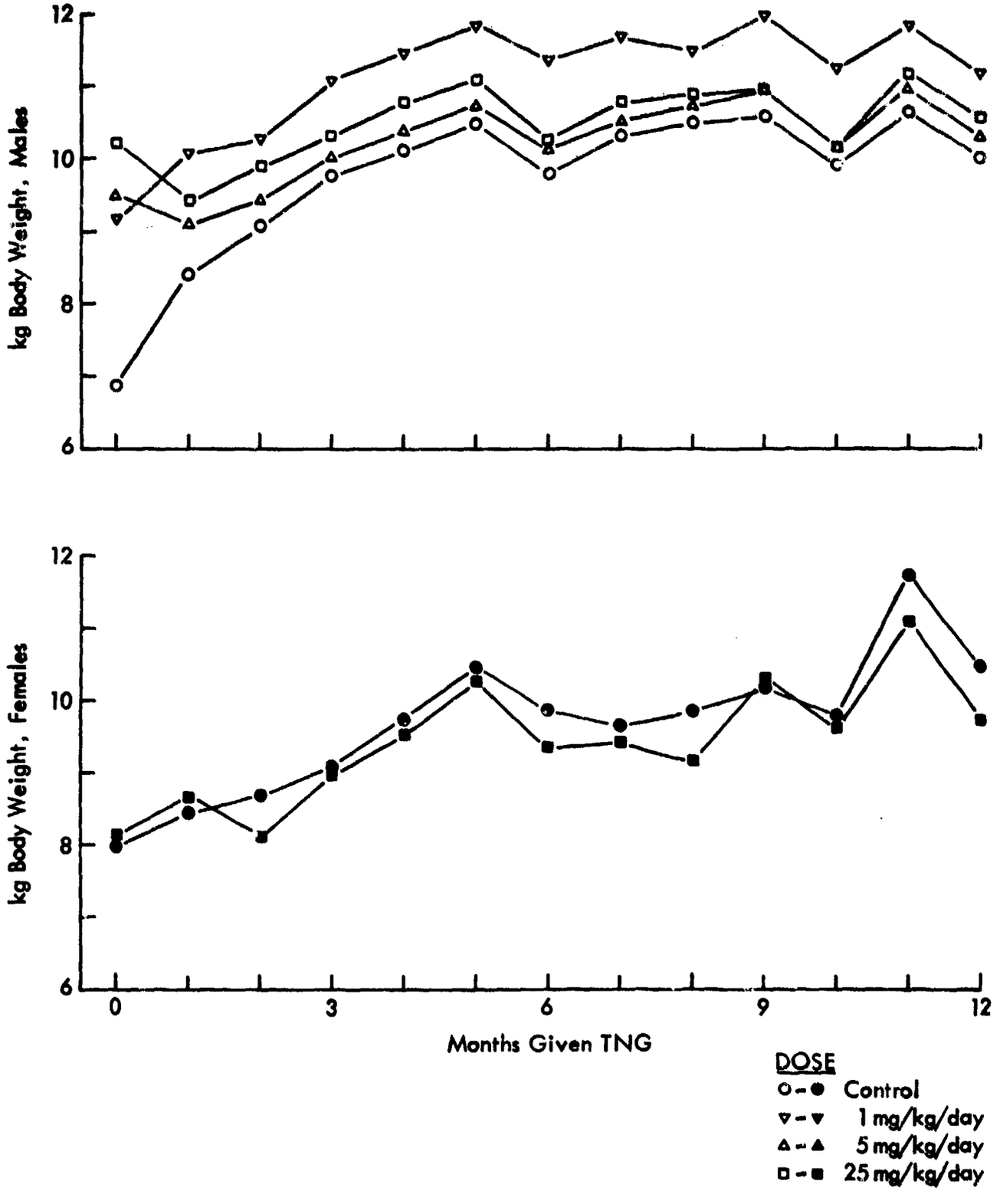

Figure 2 - Average Body Weights of Dogs Given TNG

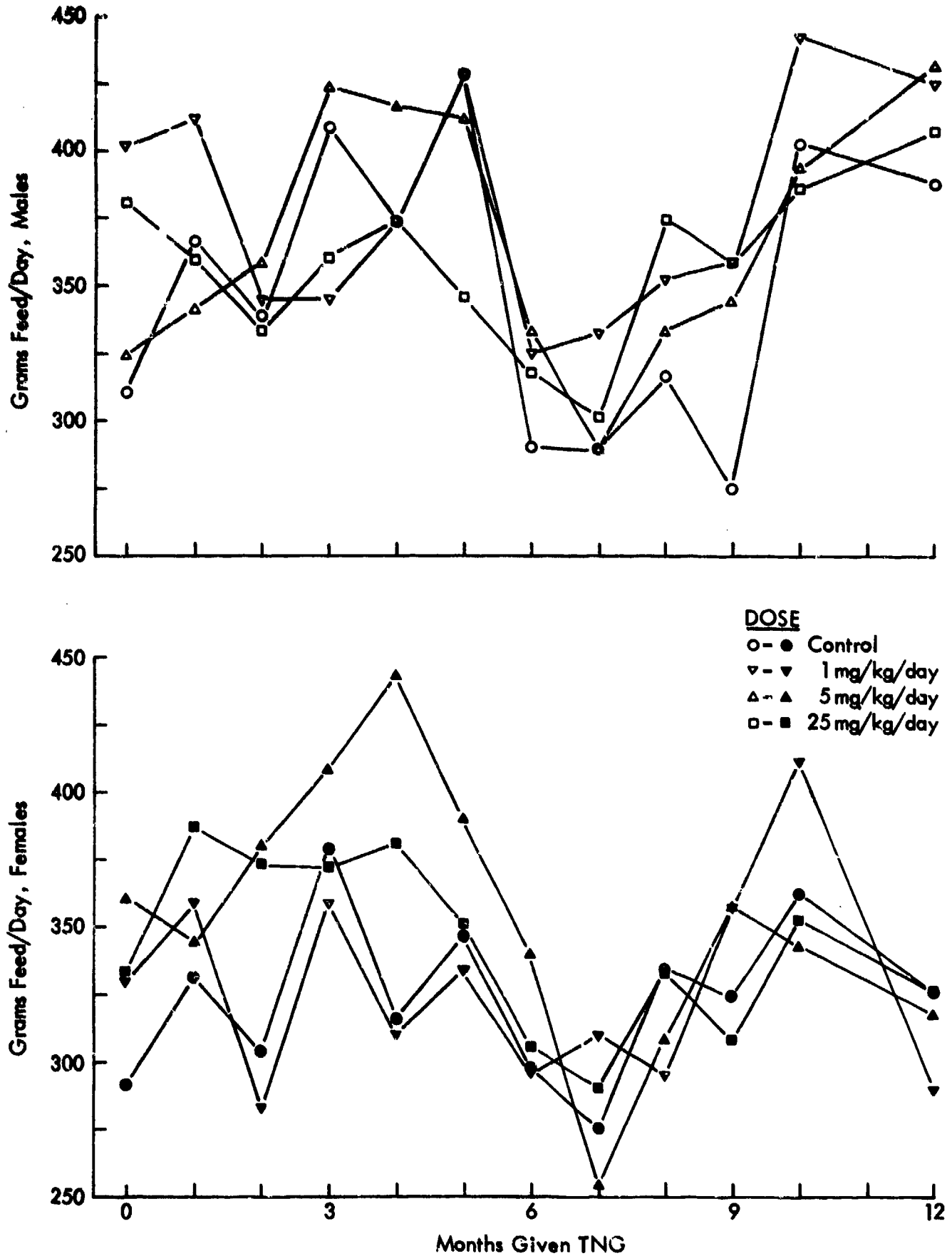

Figure 3 - Average Feed Consumption of Dogs Given TNG

IV. RAT STUDIES

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IV. RAT STUDIES

The results and interpretation of the studies in rats are described below.

A. Observations and Toxic Signs

There were few toxic signs observed. By month 3, the high-dose (1% TNG in feed) rats were obviously underweight, as discussed below. Furthermore, their fur was usually a dirty tan color. There were no obviously localized stainings, but the rough, matted appearance showed that this was due to a lack of grooming. Occasionally, some of the high-dose rats would have bluish skin, especially around the snout; we ascribe this to methemoglobinemia.

As usual, there were unscheduled deaths, summed in Figures 4 (males) and 5 (females). All the groups were generally similar, except the high-dose females, who had a conspiciously lower death rate. For lack of any other cause, we tentatively ascribe this to decreased obesity. The major causes of death were pituitary adenomas, ulcerated subcutaneous tumors, and miscellaneous causes. Cases will be discussed in turn.

The most common background tumor in this strain of rats is the pituitary chromophobe adenoma,[16] which accounted for a majority of the unscheduled deaths. We could usually identify the rats with this tumor by observing their motor function. Most commonly, they had unilateral ataxia or paralysis. Sometimes this was seen in the hindquarters, sometimes over the entire body. In addition, many of these rats became hyperexcitable, showing exaggerated motor responses to stimuli. Other signs seen at times were tear pigments around the eyes or snout and exophthalmos. Severe tumors were accompanied by large weight losses; a loss of 100 g or more within 2 weeks identified moribund rats for necropsy. One of the earliest cases was middle-dose (0.1% TNG) female No. 132, who lost over 200 g in 3 weeks. Her terminal blood (see Table 17) was somewhat concentrated (perhaps due to dehydration from decreased drinking), but otherwise normal.

Subcutaneous tumors were quite common at milkline sites in females and sometimes seen at other sites and in males. The faster growing tumors, identified by repeated observations and the tautly stretched skin above them, often became ulcerated. Then, the rat would be killed for necropsy, as the low-dose (0.01% TNG) female No. 81-212 was when a tumor beside her left front leg became ulcerated.

Some of the miscellaneous deaths were readily diagnosed. For instance, low-dose male No. 81-102 was unusually obese (over 800 g weight and still increasing) when he was bled after 6 months feeding. In the process,

he suffocated in the holder when his attempts to escape caused his mass of fat to block air circulation. He was replaced by an extra male who had received the low-dose feed, but not been weighed regularly. During week 44, low-dose metabolism male No. 81-415 had rales and appeared to have lost weight. He was not weighed regularly. We feared infectious disease, and killed him for necropsy. His blood was normal (Table 17); pathology is listed below. One of the more interesting rats was high-dose metabolism male No. 83-431. When killed in week 62, he was weak, especially in the hindquarters, similar to the rats with pituitary adenomas. His skin was very loose, implying recent weight loss. The gross examination found extreme splenomegaly (10.0 g) and prominent, greenish thymus and lymph nodes in an emaciated body. The laboratory data (Table 17) were bizarre. The tabulated 94% bands (immature granulocytes) are misleading. The actual observations were 14% bands, 25% metamyelocytes, 26% myelocytes, 26% promyelocytes, and 3% myeloblasts, representative of the entire hematopoietic pathway. The eosinophils were actually eosinophilic metamyelocytes. The few erythrocytes present had moderate anisocytosis and poikilocytosis, with lesser incidence of other abnormalities. The serum glucose and enzyme levels show gross derangements of liver function, including complete failure of gluconeogenesis (a sign of terminal starvation in humans). Since this was the only case of such extreme leukemia in any dose group, we believe it is a incidental lesion not related to TNG feeding.

However, some of the unscheduled deaths had no obvious cause. The most common symptom was inanition. Sometimes, this accompanied rapid tumor growth, with the tumor apparently starving the body of necessary nutrients. Some deaths occurred at night and autolysis hindered or prevented necropsy. Laboratory data from selected rats are included in Table 17; included are rats with abnormal findings and high-dose rats.

B. Body Weight

Average body weights of rats fed various doses of TNG are shown in Figure 6. Control rats gained weight quickly at first, and then at a decreasing rate. Male rats reached a plateau of over 800 g after about 15 months. Females reached a plateau of over 500 g near the end of the study. As usual, there were month-to-month fluctuations, especially in the last months as rats had weight losses from inanition or pituitary adenomas, or weight increases from subcutaneous tumors, and then died. Growth rate of the low-dose rats was about the same as controls throughout the study; much of the curve is omitted from the graph for clarity. Growth rate of the middle-dose rats was about the same as controls at first. After 3 months (males) or 12 months (females), they weighed consistently less (about 60 g in males and 30 g in females) than controls. The body weights of high-dose males significantly lagged behind those of the controls immediately after the starting of treatment. The high-dose females lost weight at first, and started to gain weight after 3 weeks. Furthermore, the body weights of the high-dose rats reached

lower plateaus, about 600 g for males and 300 g for females, considerably less than those of the controls.

C. Feed Consumption and TNG Intake

The average feed consumptions are shown in Table 18. To provide equal weighting on the time scale, the weekly measurements in the first month were averaged to create a composite value for the month. There is a dose-related decrease in feed consumption among the males and females, although the differences are not statistically significant, with the exception of difference between the high-dose females and the controls. This decreased consumption is most extreme in the first 3 weeks, as shown in Table 19.

The average TNG intakes are also shown in Table 18; the monthly intakes are plotted in Figure 7. The TNG intake values were corrected for TNG evaporation. Evaporation was determined by assaying TNG content of feed in a cage (without animals) over the period of a week. The initial TNG intakes were similar between the sexes. As the males gained weight faster (Figure 6), their average TNG intake, based on kilogram body weight, decreased faster. The high-dose rats had drastically decreased feed consumption in the first weeks of the study (Table 19). Shortly thereafter, both the males and females increased their feed consumption with increased TNG intakes. The fluctuations reflect the usual month-to-month biological variation in feed consumption. The overall averages of TNG intake by the low, middle and high males were 3.04, 31.5, and 363 mg/kg/day, respectively; and those of the females were 3.99, 38.1 and 434 mg/kg/day, respectively.

D. Laboratory Data

Baseline hematologic data for the various groups of male and female rats are shown in Tables 20 and 21, respectively. The values for various parameters were normal, with only toxicologically insignificant differences between groups.

Laboratory data after 3, 6, 9, 12, 18 and 24 months of feeding TNG are shown in Tables 22 through 33. The most consistent effect seen was methemoglobinemia (typically 10 to 30% of total hemoglobin in the individual rats) in the high-dose rats. Small amounts of methemoglobin were occasionally seen in other dose groups, but this is an artifact of the assay method, which involves the difference of two absorbance readings (see Appendix I). By 24 months the high-dose rats had apparently acclimated to the treatment. Only a negligible amount was found in one female rat. During the first months of the study, the high-dose rats had an elevated erythrocyte count, hematocrit and hemoglobin, which are interpreted as hemoconcentration. Toxic methemoglobinemia gives rise to anemia and a compensatory increase in erythropoiesis.[14,15] The only evidence of this response was a variable incidence

of reticulocytosis in the high-dose rats. Apparently, normal mechanisms compensated for this toxicity. The high-dose males after 24 months dosing (Table 32) showed a small decrease in glucose and an increase in the serum enzymes, a phenomenon seen in most of the later unscheduled deaths of high-dose rats (Table 17).

Laboratory data from rats fed TNG for 12 months and allowed to recover for 1 month (Tables 34 and 35) showed no methemoglobinemia. The only effects seen in the rats fed 24 months and allowed to recover (Tables 36 and 37), were abnormal values in the clinical chemistry parameters for a glucose level of 20 mg %, alkaline phosphatase of 214 IU/liter, SGOT of 2300 IU/liter, and SGPT of 1330 IU/liter.

E. Pathology

Data are available on 31 male and 36 female control rats, 28 and 37 low-dose rats, 33 and 36 middle-dose rats and 27 and 33 high-dose rats. In addition, data are included on one male and one female control rats, six male and three female low-dose rats and two male high-dose rats from the metabolism study which died at unscheduled times. Missing data are due to autolysis, cannibalism, etc.

1. Feeding for 12 Months

a. Organ Weights

Absolute and relative organ weights of rats fed TNG for 12 months are listed in Table 38. The high-dose rats had decreased body weight reflected in organ weights relative to body weight that were generally significantly higher than those of the controls. In addition, these high-dose rats had enlarged livers. The high body weight of middle-dose females and the high kidney weight of the high-dose females were normal variations. They were not seen elsewhere. After rats were allowed to recover on control feed for a month, the organ weights are essentially the same (Table 39). The statistically significant increase in spleen weights of the high-dose males was a normal variation.

b. Tissue Lesions

Tissue lesions in the male and female rats fed TNG for 12 months are summarized in Tables 40 and 41, respectively, those from the rats allowed to recover for a month are summarized in Tables 42 and 43. No recovery was apparent, the lesions are considered together.

(1) Naturally Occurring Lesions

A number of spontaneous lesions were found in various tissues of these rats, mostly mild degenerative changes. The lesions either were occasionally seen in some rats, or occurred in both the control and the treated rats.

(2) Treatment-Related Lesions

Two distinct liver lesions were found. The first was cholangiofibrosis, proliferation of the bile ducts and fibrous tissue, in high-dose rats. Grossly, there were white patches of various sizes scattered across the liver (Figure 8). Microscopic examination showed various degrees of development, from mild to severe (Figures 10 and 11) in these rats. This lesion was more severe in the rats allowed to recover for 1 month. This may represent merely varying susceptibility, but the additional time for the lesions to develop in the recovery study is also a factor.

The second lesion was a progressive development of hepatocellular carcinoma, first described by Reuber[17] for N-2-fluorenyldiacetamide and by Newberne and Wagan[18] for aflatoxin B_1. Nomenclature was clarified by a National Cancer Institute-sponsored workshop,[19] and was followed in this report. The initial stage was foci (smaller lesions) or areas (lesions of lobule size or smaller) of altered hepatocytes. These have been called "hyperplastic foci" or similar terms in earlier reports. Liver architecture was preserved and there was no clear-cut demarcation between affected and nonaffected cells. This lesion was seen in two control rats, six low-dose rats and 10 middle-dose rats. This lesion and/or the later stages was seen in all high-dose rats but one. Severity was similarly dose-related, with only mild lesions in control and low-dose rats, mild or moderate in middle-dose rats, and all degrees from none to severe in the high-dose rats.

The next stage was neoplastic nodules, formerly called hyperplastic nodules. These were spherical lesions, as large as several lobules, without normal internal architecture. A useful criterion was the presence of a sharp border with compression of the normal liver tissues immediately outside the nodule. This lesion was seen in several high-dose rats and, in mild degree, in one middle-dose rat (No. 82-322). The last stage was hepatocellular carcinoma, seen only in high-dose male No. 83-326. Pathology will be more fully described below, with the other cases. The workshop[19] concluded that all rat hepatic cell tumors had the potential for malignant behavior. Therefore, the term "hepatoma" was discarded and all tumors were labeled "carcinomas."

There was excessive pigmentation in the spleens of all high-dose rats and the epithelium of the kidney of many, including most of the females. Some mild cases were seen in other treated rats. This pigment was in the form of brownish granules, resembling hemosiderin. However, it was distinguished by a weak or missing Prussian blue reaction, indicating little or no iron.

2. Feeding for 24 Months Including Unscheduled Deaths

a. Organ Weights

Absolute and relative organ weights of rats fed TNG for 24 months are listed in Table 44. The various values were much like that seen after 12 months feeding. High-dose rats had decreased body weights causing increased relative organ weights. Some normal variations included low body weight in middle-dose females and low heart weight in high-dose females. The results in rats allowed to recover for 1 month were similar (Table 45), although somewhat obscured by the rats dying at unscheduled times including all three low-dose males.

The high-dose rats had remarkably large livers. Of 11 high-dose male rats, all had livers weighing over 30 g (up to 167.40 g for No. 83-173), while only one of the other 24 males had a liver over 30 g (middle-dose No. 82-137 having a liver weighing 43.36 g). Of the 21 high-dose female rats, only one had a liver weighing less than 22 g (No. 83-286 in the recovery study having a liver weighing 18.24 g), while only one of the other 33 females had a liver weighing more than 22 g (low-dose No. 81-224, having a liver weighing 22.52 g).

b. Tissue Lesions

Tissue lesions in rats fed TNG for 24 months are summarized in Tables 46, 47 and 48, and in rats allowed to recover for 1 month in Table 49. Lesions in rats that died or were terminated at unscheduled times are summarized in Tables 50 through 56. These results do not include all animals or all organs because some rats died at night, and autolysis hindered examination. To increase the numbers available for calculating incidence, we included all rats fed the same dosage mixtures used in the various studies. Rats with numbers in the 300's were intended to be fed for 12 months, but died early; rats with numbers in the 400's were intended for the metabolism study.

(1) Naturally Occurring Lesions

A great variety of naturally occurring lesions were found in these geriatric rats. The lesions were listed in the lower part of the appropriate tables. There were two classes of lesions: the degenerative lesions found in most geriatric rats and the rare lesions found in a few scattered rats. Typical degenerative lesions included mild chronic murine pneumonia (endemic among rats) and mild simple bile duct hyperplasia. The rare lesions included a variety of tumors and lesions in various tissues. The various tumors were extracted from Tables 46 through 56 and listed in Table 57. No obvious dose relationship existed among these relatively rare tumors or lesions; they were not related to the treatment.

(2) Treatment-Related Lesions

As seen in rats fed TNG for 12 months, a number of treatment-related lesions occurred and were listed in the upper part of the appropriate tables. The incidence of these TNG-related lesions was extracted from Tables 46 through 56, including all rats fed for more than a year and the few earlier unscheduled deaths, and tabulated in Table 58. Statistical analysis is Chi-square tests or exact probabilities on contingency tables of these data with $p < 0.05$ considered significant.

As seen in Table 58, the liver lesions occurred earlier, but were much more advanced. Almost all high-dose rats had cholangiofibrosis, usually severe. Besides the cholangiofibrosis, some livers had nonfibrous bile duct hyperplasia, cystic, adenomatoid, or both. Some of these cystic livers looked as if the rat was attempting to grow a gallbladder. In many cases, this liver lesion was combined with a progressive development of hepatocellular carcinoma. These lesions were responsible for the enormous liver sizes, as shown in the gross photograph (Figure 9). Photomicrographs showed advanced cholangiofibrosis (Figure 14) and one variety of the carcinoma (Figure 15). A usual variety of carcinoma architecture[19] was seen in these rats, ranging from regular bi-layer to random agglomerations. Hepatocellular carcinomas occurred in some middle-dose rats but the incidence was less. The middle-dose rats also had a significant increase in the incidence of areas of hepatocellular alteration, the first step in liver carcinogenesis.

Occasional metastatic nodules were seen in the lungs of some rats (Table 57). These were identifiable as originating in the hepatocellular carcinoma (see Figure 17).

Half the high-dose males had interstitial cell tumors in the testis (Figure 13). The growth of the tumor within the tunica albuginea produced pressure on the tubules, causing atrophy and aspermatogenesis. This atrophy occurred rarely in nontumorous testes from rats of all dosage groups, and is not direct toxic effect of TNG. Interstitial cell tumors were also seen in a few lower dose and control rats.

As seen in rats fed TNG for 12 months, the high-dose rats, especially the female, had increased pigment deposits in the spleen and the renal epithelium. These pigments resembled hemosiderin, and are presumably hemoglobin-derived.

One most unusual effect was a considerable decrease in the incidence[16/] of the two most common tumors in the high-dose rats: pituitary adenoma in both males and females and mammary tumors, primarily fibroadenomas in the females. The mechanism of this decrease is unknown. The decrease is probably responsible, at least in part, for the increased life span seen in high-dose females. These tumors were the most common causes of death.

Hepatic hemangiosarcomas were seen only in three high-dose males. This is not statistically significant ($p = 0.09$), and may represent normal variations.

F. Three-Generation Reproduction Study

As shown in Table 59, the mean body weights of both male and female high-dose rats were significantly decreased at all matings. No specific effects on the fertility of the F_o generation were seen. However, the F_1 generation of high-dose rats had severely impaired fertility. The first mating of the F_1 generation produced only three litters, the F_{2a}. None of the F_1 dams which littered from the first matings, had a second litter (F_{2b}). Therefore, first litters (F_{2a}) from the second mating were used to produce the F_3 litters. Although the 14 pairs of F_{2a} rats were mated twice, there was only one F_3 litter. The high-dose F_{2a} females were then mated for a third time with control males. We found that 13 of 14 became pregnant. It was then evident that the infertility of F_{2a} generation was due to the males. This conclusion was strengthened by the observation that F_{2a} high-dose males had very small testes (about one-fourth normal size) and that they produced a high incidence of vaginal plugs without sperm. In addition, microscopic examination revealed severe aspermatogenesis and mild to moderate increased interstitial tissue in the testes of these F_{2a} males.

The data for the various litters are detailed in Table 60. All parameters (litter size, live-born index, birth weight, viability and lactation indexes and weaning weight) except male ratio, were reduced in the high-dose F_{1a} litters. Most parameters were also reduced to some extent in the high-dose F_{1b} and F_{2a} litters. These adverse effects of the high dose of TNG appeared to be secondary to the poor nutritional status of the dams. During the F_{1b} gestation period, feed intake by the dams was measured. The feed intake of these high-dose F_{1b} rats was about 65% of those of the controls. The product of the litter size and birth weight, that is, total litter weight can be considered as gestational product. It is interesting to note that the gestational product of the high-dose F_{1b} rats was about 62% of those of the controls.

The reduction in litter size of the high-dose F_{1a} and F_{1b} litters might suggest mutagenic and/or teratogenic effects. The high-dose F_0 males and females were mated with control rats following the general procedure in Appendix II. The results of the dominal lethal mutagenic study of the males are discussed below (Section G.2.).

Results of the teratogenic study (the third mating of the females of the F_0 generation) are given in Table 61. The weight of the high-dose females on gestation day 0 and their weight change (excluding the uterus and contents) were significantly less than the control group. In addition, their liver weights were significantly increased relative to their corrected body weight. The number of implants and the viability of these implants were not affected. The mean fetal weight was not reduced in comparison with the concurrent control group. Examination of the soft tissues of one-half of the fetuses disclosed only a single malformation in the high-dose group which appeared to be related to the TNG exposure. Although the incidence of diaphragmatic hernia was not judged significant by the two-sample rank test, its occurrence in four of 19 litters of the high-dose group and the neonatal death for the F_{1a} and F_{1b} litters in the three-generation study suggest this teratogenic effect as a possible cause of the reduced litter sizes observed with the earlier litters. Examination of the skeleton of the other half of the fetuses revealed a single site of anomalies in the high-dose group which appeared to be related to the TNG exposure. The incidence of absent and incomplete ossification of the hyoid bone were significantly increased when compared to controls. The skeletal anomalies are indicators of delayed development and are not normally considered as valuable in assessing teratogenic potential. However, the sternabra, centrum and bones of the skull were not similarly affected.

G. Mutagenesis Studies

1. Cytogenetic Study

The results of the chromosome analysis of the bone marrow and kidney cultures from rats fed TNG for 24 months are shown in Tables 62 and 63. There were no statistically significant changes. The only slight difference was increased chromatid breaks and gaps in the kidney cultures. Since "these aberrations are of questionable relevance with regard to heritable events of importance to man,"[20] they are not considered toxicologically important.

2. Dominant Lethal Mutation Study

The results of the dominant lethal mutation study, using males from the main chronic toxicity study mated to untreated females after their (the males) second mating to treated females in the three generation reproduction study, are shown in Table 64. There was no evidence of an adverse effect on

male fertility, on preimplantation loss as indicated by the implantation index, or on postimplantation losses as indicated by the implant viability index. There was no dominant lethal mutation effect. Furthermore, the reduced litter sizes seen by these high-dose males and the high-dose females of the three-generation reproduction study (Table 60) are apparently due to non-genetic factors.

H. Metabolism Studies

The results of the metabolism of TNG in rats fed TNG for 3, 12 or 24 months are shown in Tables 65 through 70. Results are similar to those seen earlier in rats not fed TNG before the metabolism study.[1] There are no consistent differences between sexes, between dose groups, or between the time periods. Radioactivity from the oral dose is excreted in the urine, air, and feces. The only major concentration within the body is in the liver, the organ of metabolism and excretion (in bile[3]). Metabolic reactions include denitrification toward glycerin, glucuronidation of various nitroglycerins, and oxidation and other reactions of the glycerin. The fate of the removed nitro groups is unknown. The urinary metabolites include little, if any, TNG, but relatively large amounts of the di- and mononitroglycerins and their glucuronides. Other major metabolites included glycerin and some unidentified polar compounds, presumably metabolites of glycerin.

I. Discussion

A general toxic effect of decreased feed consumption causing decreased weight gain was seen in rats fed the high dose of TNG. Interestingly, this was not accompanied by a decreased life span. In fact, the high-dose females, although most affected in certain systems (such as liver), had the lowest death rate.

Profound methemoglobinemia occurred in the high-dose rats. The only sequelae were occasional reticulocytosis, indicating an anemia corrected by the natural erythropoietic mechanisms, and deposits of hemoglobin-derived pigment seen in the spleens and/or the renal epithelium of some high-dose rats.

Two distinct types of lesions were found in the livers of the high-dose rats. Almost all of them had hyperplasia of the bile ducts, often highly fibrous, known as cholangiofibrosis. In addition, there was a progressive development of hepatocellular carcinoma, following a pattern seen with chemically distinct compounds such as N-2-fluorenyldiacetamide[17] and aflatoxin B_1.[18] These changes eventually caused considerable elevations of serum enzyme levels. The incidence of foci and areas of hepatocellular alteration,

the first stage in the development of hepatocellular carcinoma, was also increased in the middle-dose rats. Male rats fed the high dose of TNG-developed interstitial cell tumors of the testis. This effect produced a secondary sterility from the pressure extended by the growing tumor on the seminiferous tubules.

The most common spontaneous tumors in the rats used for the study are pituitary chromophobe adenomas and mammary tumors, especially fibroadenomas.[16] The high-dose rats had a significant decrease in these tumors. These decreases in tumor incidence contributed to the observed decrease in death rate.

The three-generation reproduction study indicated severely impaired fertility in the F_1 and F_2 generation of the high-dose males. Microscopic examination of the F_2 males revealed severe aspermatogenesis and mild to moderate increased interstitial tissue in the testis. Various parameters measuring the F_1 and F_2 litters were reduced due to, at least in part, poor nutritional status of the dams. There were no significant effects on chromosomes in the cytogenetics study and no mutagenic effect in the dominal lethal study. Continual feeding of TNG did not affect how the body absorbed, metabolized and excreted a large oral dose of the compound.

J. Conclusions

The high dose, with TNG intake of 363 mg/kg/day in males and 434 mg/kg/day in females, was toxic, decreased feed consumption and depressed weight gain. Target organs included the blood (methemoglobin), liver (cholangiofibrosis and hepatocellular carcinoma) and testis (interstitial cell tumor). There was a decrease in the naturally occurring tumors of the pituitary and mammary gland, which decreased the death rate in females. The three-generation reproduction study indicated severely impaired fertility of the high-dose F_1 and F_2 males. No specific effects were seen on chromosomes in the cytogenic study, on mutagenesis in the dominal lethal study, or on the metabolism of TNG. This toxic dose in rats is enormously larger than the usual human dose range of 200 μg to 10 mg/man/day.[21]

The middle dose, with TNG intake of 31.5 mg/kg/day in males and 38.1 mg/kg/day in females, was slightly toxic. It produced the preliminary lesions which can develop into hepatocellular carcinoma.

The low dose, with TNG intake of 3.04 mg/kg/day in males and 3.99 mg/kg/day in females, had no apparent toxic effects.

TABLE 17

LABORATORY DATA OF RATS FED TNG AND DYING AT UNSCHEDULED TIMES

Dose (% in feed):	0.01	0.01	1	0.1	0.01	0.01	0.1	1	1	1	1	0.1	1	1	0.01	0.1	1
Rat No.:	212	415	431	132	121	204	152	186	284	164	273	146	180	181	218	259	432
Week of Death:	40	44	52	70	80	80	80	83	85	88	84	97	97	97	97	98	103
Erythrocytes, x $10^6/mm^3$	5.42	7.27	3.07	8.78	2.28	2.04	6.91	5.60	5.81	7.51	4.71	6.09	--[a]	5.99	6.53	4.71	6.09
Heinz bodies, %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	0.00	0.00	0.00	0.00
Reticulocytes, %	0.32	0.86	2.63	0.54	12.2	19.3	0.74	2.24	3.05	2.94	6.69	1.72	--	1.07	0.32	13.11	0.64
Hematocrit, vol %	36	48	13	49	17	18	44	38	37	43	36	34	--	42	43	33	39
Hemoglobin, gm %	11.6	15.4	5.4	17.6	5.8	5.8	14.3	11.5	11.7	14.2	10.2	12.7	--	13.1	15.8	11.9	12.6
Methemoglobin, %	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	7.0	9.0	0.0	--	5.3	0.0	4.2	13.4
MCV, cubic microns	66.4	66.0	42.3	55.8	74.6	88.2	63.7	67.9	63.7	57.3	76.4	55.8	--	70.1	65.8	70.1	64.0
MCHB, micromicrograms	21.4	21.2	17.6	20.0	25.4	28.4	20.7	20.5	20.1	18.9	21.7	20.9	--	21.7	24.2	25.3	20.7
MCHBC, gm %	32.2	32.1	41.5	35.9	34.1	32.2	32.5	30.3	31.6	33.0	28.3	37.4	--	31.2	36.7	36.1	32.3
Platelets, x $10^5/mm^3$	4.95	5.35	1.50	7.75	11.10	8.50	6.90	2.75	5.60	5.65	5.35	4.15	--	5.55	5.00	5.05	3.10
Leucocytes, x $10^3/mm^3$	4.0	5.1	381.6	5.9	7.6	19.2	8.0	11.4	9.2	5.1	8.0	6.1	--	13.9	5.7	4.4	6.2
Neutrophils, %	25	25	2	42	60	46	45	41	30	31	61	71	--	47	65	35	30
Lymphocytes, %	73	75	1	56	40	52	59	49	56	70	69	29	--	53	35	64	68
Bands, %	0	0	94	0	0	0	0	0	0	0	0	0	--	0	0	0	1
Monocytes, %	1	0	0	0	0	0	0	3	1	0	0	0	--	0	0	0	0
Eosinophils, %	1	0	3	2	0	2	3	2	0	0	0	0	--	0	0	1	1
Basophils, %	0	0	0	0	0	0	0	0	0	0	0	0	--	0	0	0	0
Atypical, %	0	0	0	0	0	0	0	0	0	0	0	0	--	0	0	0	0
Nucleated RBC, %	0	0	1	0	0	18	0	0	0	0	0	0	--	0	0	0	0
Glucose, mg %	154	122	10	119	113	116	104	116	115	105	105	91	63	77	72	120	94
SGOT, IU/ℓ	43	55	759	80	55	65	93	68	62	43	74	74	347	592	130	55	46
SGPT, IU/ℓ	24	31	124	46	24	28	21	31	37	28	24	52	108	167	49	31	28
Alkaline phosphatase, IU/ℓ	29	64	285	13	106	47	33	48	31	59	38	79	125	63	12	186	34
BUN, mg %	15	11	21	12	180	11	14	15	19	17	16	275	25	15	63	26	12
IgE, IU/ℓ	--	<500	2250	--	--	--	--	1900	<450	<425	<425	--	425	425	--	--	<450

[a] Sample clotted.

TABLE 18

FEED CONSUMPTION AND COMPOUND INTAKE OF RATS
FED TNG FOR 24 MONTHS

Dose (% in feed)	Males		Females	
	Feed Consumption (g/rat/day)	TNG Intake (mg/kg/day)	Feed Consumption (g/rat/day)	TNG Intake (mg/kg/day)
0	26.79 ± 1.05[a/]	--	19.30 ± 0.92	--
0.01	26.65 ± 0.82	3.04 ± 0.16	19.99 ± 0.44	3.99 ± 0.18
0.1	25.90 ± 0.68	31.5 ± 1.6	18.56 ± 0.49	38.1 ± 1.6
1	23.77 ± 0.67	363 ± 10	15.07 ± 0.50[b/]	434 ± 11

a/ Mean ± standard error of 24 measurements; the first month is the average of three measurements.

b/ Significantly different from control by Dunnett's multiple comparisons procedures.

TABLE 19

FEED CONSUMPTION OF RATS
FED TNG FOR 3 WEEKS

Dose (% in feed)	Feed Consumption (g/rat/day) Males	Females
0	24.4 ± 0.7[a/]	16.4 ± 0.4
0.01	24.3 ± 1.0	17.0 ± 0.4
0.1	23.2 ± 0.7	14.9 ± 0.2
1	16.4 ± 2.2[b/]	9.0 ± 2.0[b/]

a/ Mean ± standard error of three measurements.
b/ Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 20

LABORATORY DATA OF MALE RATS BEFORE FEEDING OF TNC

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	0.0 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.13 ± .23	6.35 ± .28	5.94 ± .15	6.04 ± .19
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	6.24 ± 1.03	6.52 ± 1.22	6.83 ± .58	6.24 ± .68
HEMATOCRIT, VOL, %	49.5 ± 1.3	51.8 ± 2.7	48.3 ± 1.5	48.8 ± 1.4
HEMOGLOBIN, GM, %	14.8 ± .5	16.4 ± .5	14.4 ± .3	14.5 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	80.8 ± 1.4	81.4 ± 1.2	81.2 ± 1.5	80.7 ± 1.1
MCHB, MICRO MICROGMS.	24.2 ± .1	25.9 ± .9	24.3 ± .3	24.1 ± .4
MCHBC, GM %	30.0 ± .4	31.9 ± 1.5	30.0 ± .5	29.9 ± .8
PLATELETS ($X10^5$ /MM^3)	5.3 ± .6	5.2 ± .3	6.6 ± .5	6.5 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	19.8 ± 3.6	20.7 ± 1.4	21.4 ± 3.6	21.4 ± .5
NEUTROPHILS, %	15.0 ± 4.3	8.8 ± .9	8.3 ± 2.7	14.0 ± 3.4
LYMPHOCYTES, %	84.3 ± 4.3	90.5 ± 1.3	91.5 ± 2.5	84.8 ± 3.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	.3 ± .3	.3 ± .3	1.0 ± .7
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .5	.5 ± .3	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 21

LABORATORY DATA OF FEMALE RATS BEFORE FEEDING OF TNG

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.07 ± .21	6.16 ± .13	6.03 ± .10	6.85 ± .23[a/]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	3.55 ± .73	4.01 ± .24	3.40 ± .23	2.78 ± .37
HEMATOCRIT, VOL, %	44.8 ± .6	45.0 ± .7	46.5 ± .6	48.3 ± 1.1[a/]
HEMOGLOBIN, GM, %	14.0 ± .5	14.1 ± .2	14.2 ± .2	15.0 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	73.9 ± 1.8	73.0 ± 1.3	77.1 ± .8	70.5 ± 1.0
MCHB, MICRO MICROGMS.	23.1 ± .4	22.9 ± .3	23.6 ± .3	21.9 ± .2
MCHBC, GM %	31.3 ± .7	31.3 ± .3	30.6 ± .2	31.1 ± .5
PLATELETS ($X10^5$ /MM^3)	7.4 ± .5	9.5 ± .9	7.2 ± .4	7.5 ± .[illegible]
LEUKOCYTES ($X10^3$ /MM^3)	14.4 ± 1.3	15.5 ± 2.2	14.0 ± 1.0	14.9 ± 1.0
NEUTROPHILS, %	6.8 ± 1.9	5.5 ± .3	8.5 ± 4.6	9.0 ± 2.8
LYMPHOCYTES, %	92.0 ± 1.7	94.3 ± .5	91.5 ± 4.6	90.5 ± 2.9
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .3	.3 ± .3	0.0 ± 0.0	.5 ± .5
BASOPHILS, %	0.0 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.8 ± .3	0.0 ± 0.0[a/]	.3 ± .3	0.0 ± 0.0[a/]

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 22

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNG FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C. 4)	0.01 (T. 4)	0.1 (T. 4)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.52 ± .14	7.43 ± .12	7.67 ± .24	8.08 ± .24
HEINZ BODIES. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.0 ± .0
RETICULOCYTES. %	.96 ± .11	1.01 ± .20	1.40 ± .14	2.46 ± .52[a/]
HEMATOCRIT. VOL. %	50.3 ± .5	52.0 ± .7	49.3 ± 1.0	56.5 ± 2.3[a/]
HEMOGLOBIN. GM. %	15.7 ± .1	16.6 ± .1	15.8 ± .4	17.3 ± .6[a/]
METHEMOGLOBIN. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	19.6 ± 4.8[a/]
MCV. CUBIC MICRONS	66.9 ± 1.1	70.0 ± 1.4	64.3 ± 1.1	69.9 ± 1.2
MCHB. MICRO MICROGMS.	20.9 ± .2	22.3 ± .5[a/]	20.6 ± .4	21.3 ± .1
MCHBC. GM %	31.3 ± .2	31.9 ± .4	32.0 ± .3	30.6 ± .3
PLATELETS ($X10^5$ /MM^3)	4.8 ± .3	5.1 ± .4	6.0 ± .6	5.2 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	16.1 ± 1.5	16.6 ± .5	18.9 ± 1.8	16.3 ± 1.7
NEUTROPHILS. %	10.3 ± .5	8.5 ± 3.0	9.8 ± 1.5	13.3 ± 3.4
LYMPHOCYTES. %	89.0 ± 1.1	90.8 ± 2.9	89.3 ± 1.5	86.8 ± 3.4
BANDS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS. %	.3 ± .3	.3 ± .3	1.0 ± 0.0[a/]	0.0 ± 0.0
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	.5 ± .5	.5 ± .3	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.5 ± .3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 23

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TNG FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C. 4)	0.01 (T. 4)	0.1 (T. 4)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.94 ± .06	6.81 ± .16	6.86 ± .11	8.10 ± .21[a/]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.8 ± .0
RETICULOCYTES, %	1.03 ± .08	1.30 ± .05	1.10 ± .17	1.14 ± .13
HEMATOCRIT, VOL. %	49.3 ± .9	45.3 ± 1.0	45.3 ± .9	56.3 ± 2.5[a/]
HEMOGLOBIN, GM. %	15.5 ± .1	14.6 ± .3	14.4 ± .3	16.9 ± .5[a/]
METHEMOGLOBIN, %	.5 ± .5	0.0 ± 0.0	0.0 ± 0.0	20.4 ± 2.9[a/]
MCV, CUBIC MICRONS	70.9 ± 1.8	66.5 ± 1.9	66.0 ± .3	69.6 ± 3.5
MCHB, MICRO MICROGMS.	22.3 ± .3	21.4 ± .4	21.1 ± .1	20.9 ± 1.0
MCHBC, GM %	31.5 ± .5	32.3 ± .4	31.9 ± .1	30.1 ± .7
PLATELETS ($X10^5$ /MM^3)	4.8 ± .2	5.4 ± .7	6.0 ± .5	5.4 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	13.5 ± 1.4	13.2 ± .8	12.4 ± 1.0	12.0 ± 1.3
NEUTROPHILS, %	5.0 ± .7	6.8 ± 2.7	5.0 ± 2.7	5.8 ± 1.3
LYMPHOCYTES, %	94.8 ± .5	93.0 ± 2.7	92.0 ± 3.9	93.5 ± 2.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	.3 ± .3	.3 ± .3	2.0 ± .7[a/]	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 24

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNG FOR 6 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C. 4)	0.01 (T. 4)	0.1 (T. 4)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.08 ± .08	6.37 ± .33	7.27 ± .37	7.23 ± .41
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.1 ± .1
RETICULOCYTES, %	.98 ± .13	1.24 ± .21	.87 ± .10	1.15 ± .18
HEMATOCRIT, VOL. %	50.8 ± .5	52.3 ± 1.3	52.0 ± 1.5	52.8 ± 1.4
HEMOGLOBIN, GM. %	15.4 ± .0	15.6 ± .3	15.4 ± .3	15.8 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	18.3 ± 2.7 a/
MCV, CUBIC MICRONS	71.7 ± 1.2	83.0 ± 6.2	71.8 ± 2.0	73.3 ± 2.6
MCHB, MICRO MICROGMS.	21.8 ± .2	24.8 ± 1.5	21.3 ± .7	22.0 ± .7
MCHBC, GM %	30.5 ± .3	30.0 ± .7	29.6 ± .2	30.0 ± .6
PLATELETS ($X10^5$ /MM^3)	6.8 ± .3	5.9 ± .3	6.2 ± .4	5.3 ± .4 a/
LEUKOCYTES ($X10^3$ /MM^3)	14.3 ± 1.3	14.5 ± 1.7	10.4 ± 1.9	13.9 ± 2.2
NEUTROPHILS, %	11.5 ± 3.1	10.0 ± 2.0	10.3 ± .9	16.0 ± 1.2
LYMPHOCYTES, %	86.3 ± 3.8	87.5 ± 2.7	88.5 ± 1.2	81.8 ± 2.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	2.0 ± .9	1.0 ± .4	1.8 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.5 ± .6	.5 ± .3	.3 ± .3	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 25

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TNT FOR 6 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.55 ± .23 (2)	7.01 ± .21	7.15 ± .18	7.45 ± .11[a/]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.78 ± .26 (2)	.99 ± .18	.94 ± .19	1.14 ± .22
HEMATOCRIT, VOL. %	49.0 (1)	47.5 ± 1.4	48.8 ± .8	53.3 ± 1.4
HEMOGLOBIN, GM. %	15.8 ± .3 (2)	15.5 ± .4	14.4 ± .3	15.7 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	17.7 ± 7.0 (3)[a/]
MCV, CUBIC MICRONS	72.3 (1)	67.9 ± 2.5	64.0 ± 1.1	71.6 ± 1.6
MCHB, MICRO MICROGMS.	24.1 ± .5 (2)	22.1 ± .9	20.2 ± .2[a/]	21.2 ± .6
MCHBC, GM %	32.7 (1)	32.6 ± .3	31.6 ± .4	29.6 ± .4
PLATELETS ($X10^5$ /MM^3)	6.5 ± .3 (3)	7.0 ± .2 (3)	7.4 ± .4	6.8 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	12.5 ± .6 (2)	8.3 ± .6	9.0 ± 1.0	10.8 ± 1.1
NEUTROPHILS, %	7.5 ± 1.5 (2)	9.3 ± 1.3	14.0 ± 2.3	14.5 ± 2.2
LYMPHOCYTES, %	92.5 ± 1.5 (2)	90.5 ± 1.3	85.8 ± 2.4	85.3 ± 2.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.3 ± .3	.3 ± .3	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 26

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNG FOR 9 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.75 ± .26	6.29 ± .36	6.88 ± .19	6.63 ± .28
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.81 ± .12 (3)	.97 ± .09	.86 ± .12	1.55 ± .14[a/]
HEMATOCRIT, VOL. %	53.0 ± .6 (3)	51.5 ± 1.2	52.8 ± 1.1	53.5 ± 1.3
HEMOGLOBIN, GM. %	15.7 ± .4	15.3 ± .1	16.0 ± .3	15.2 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.8 ± 2.5[a/]
MCV, CUBIC MICRONS	75.7 ± .4 (3)	82.6 ± 4.3	76.7 ± 1.5	81.1 ± 3.5
MCHB, MICRO MICROGMS.	23.3 ± .5	24.5 ± 1.4	23.4 ± .5	22.9 ± .2
MCHBC, GM %	30.3 ± .6 (3)	29.7 ± .6	30.4 ± .1	28.4 ± 1.0
PLATELETS ($X10^5$ /MM^3)	6.1 ± .1	5.2 ± .4	5.9 ± .3	5.3 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	19.5 ± 3.9	17.1 ± 2.3	18.2 ± 1.8	18.8 ± 2.7
NEUTROPHILS, %	13.8 ± 1.9	15.8 ± 3.5	12.0 ± 1.8	21.3 ± 7.0
LYMPHOCYTES, %	86.3 ± 2.4	82.0 ± 3.2	85.0 ± 2.0	76.3 ± 6.6
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .6	1.3 ± .5	2.0 ± .4	1.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	1.0 ± 0.0	1.0 ± .4	.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 27

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TBG FOR 9 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.71 ± .31	5.28 ± .24	5.41 ± .16	5.75 ± .11
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.81 ± .11 (3)	1.09 ± .15	1.08 ± .10 (3)	1.35 ± .17 (3)
HEMATOCRIT, VOL, %	47.3 ± 2.7	46.8 ± .9	44.8 ± 2.1	48.0 ± 1.7
HEMOGLOBIN, GM, %	15.2 ± .8	14.3 ± .3	13.5 ± .3	14.4 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1.3 (3)
MCV, CUBIC MICRONS	83.8 ± 8.1	88.9 ± 2.9	82.7 ± 2.5	83.4 ± 2.2
MCHB, MICRO MICROGMS.	26.6 ± .7	27.3 ± .9	25.0 ± .5	25.1 ± .3
MCHBC, GM %	32.4 ± 2.6	30.7 ± .4	30.4 ± 1.4	30.1 ± .6
PLATELETS ($X10^5$ /MM^3)	5.2 ± .3	5.5 ± .4	6.9 ± .3[a/]	5.4 ± .6
LEUKOCYTES ($X10^3$ /MM^3)	15.7 ± 1.4	15.4 ± 1.1	12.8 ± 1.2	15.4 ± 2.1
NEUTROPHILS, %	16.0 ± 5.4	15.3 ± 2.1	10.7 ± 1.2 (3)	16.5 ± 2.4
LYMPHOCYTES, %	81.5 ± 5.5	82.3 ± 1.9	88.0 ± .6 (3)	81.0 ± 2.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± .5	1.3 ± .5	.8 ± .8	2.0 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.3 ± .8	1.3 ± .6	.3 ± .3	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 28

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNG FOR 12 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.68 ± .15	7.08 ± .21	7.98 ± .23	7.91 ± .23
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.38 ± .12	.39 ± .04	.37 ± .03	1.45 ± .25*/
HEMATOCRIT, VOL. %	54.3 ± .8	51.0 ± .9	55.5 ± .3	52.8 ± 1.9
HEMOGLOBIN, GM. %	16.8 ± .3	16.2 ± .4	16.7 ± .2	15.7 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.8 ± 2.9*/
MCV, CUBIC MICRONS	70.7 ± 1.0	72.2 ± 1.6	69.7 ± 2.1	66.6 ± 1.0
MCHB, MICRO MICROGMS.	21.9 ± .3	22.9 ± .9	21.0 ± .7	19.8 ± .3
MCHBC, GM %	30.9 ± .4	31.8 ± .5	30.2 ± .4	29.7 ± .3
PLATELETS ($X10^5$ /MM^3)	7.4 ± .4	5.1 ± .2	7.8 ± 1.1	7.4 ± .9
LEUKOCYTES ($X10^3$ /MM^3)	16.9 ± 2.0	15.3 ± 2.3	16.2 ± 1.5	20.7 ± 3.4
NEUTROPHILS, %	14.5 ± 2.6	14.5 ± 4.0	21.3 ± 6.4	30.8 ± 6.6
LYMPHOCYTES, %	83.8 ± 2.0	84.5 ± 4.0	76.8 ± 6.3	68.0 ± 6.9
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± 1.0	.5 ± .3	1.5 ± .3	.5 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.5 ± .3	.5 ± .3	.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	124.3 ± 3.3	129.0 ± 10.7 (3)	128.3 ± 4.6 (3)	117.8 ± 8.1
SGOT, IU/L	167 ± 99	68 ± 8 (3)	55 ± 4 (3)	288 ± 216
SGPT, IU/L	118 ± 84	32 ± 8 (3)	28 ± 2 (3)	219 ± 187
ALK. PHOS., IU/L	56 ± 10	45 ± 2 (3)	26 ± 7 (3)	52 ± 15
BUN, MG %	14.3 ± 2.1	14.7 ± 1.5 (3)	14.3 ± 1.2 (3)	15.3 ± 2.3
IMMUNOGLOBULIN E, IU/ML	2663 ± 583			1463 ± 385

ENTRIES ARE MEAN ± STANDARD ERROR

*/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 29

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TNG FOR 12 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.76 ± .08	6.94 ± .16	6.79 ± .14	6.17 ± .40 (3)
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.38 ± .08	.86 ± .12	.52 ± .12	.19 ± .03 (3)
HEMATOCRIT, VOL. %	45.5 ± .9	48.0 ± .9	45.5 ± .6	45.3 ± 4.1 (3)
HEMOGLOBIN, GM. %	14.8 ± .3	15.1 ± .5	14.4 ± .1	14.1 ± 1.1 (3)
METHEMOGLOBIN, %	.4 ± .4	.4 ± .4	0.0 ± 0.0	13.2 ± 6.5[a/]
MCV, CUBIC MICRONS	67.3 ± .8	69.3 ± 1.4	67.1 ± 1.4	73.3 ± 2.4 (3)
MCHB, MICRO MICROGMS.	21.9 ± .3	21.8 ± .4	21.3 ± .4	22.8 ± .6 (3)
MCHBC, GM %	32.5 ± .7	31.4 ± .4	31.7 ± .2	31.1 ± .3 (3)
PLATELETS ($X10^5$ /MM^3)	5.1 ± .7	7.2 ± .7	5.9 ± .6	6.3 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	10.0 ± .5	12.4 ± .1	11.5 ± .8	11.3 ± .9 (3)
NEUTROPHILS, %	14.5 ± .9	18.5 ± 1.9	15.3 ± 2.1	24.0 ± 2.5[a/]
LYMPHOCYTES, %	84.5 ± .6	80.0 ± 2.0	83.3 ± 2.4	74.3 ± 1.8[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	1.5 ± .3	.8 ± .5	1.3 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	0.0 ± 0.0	.8 ± .3	.5 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	111.3 ± 5.3	114.8 ± 6.6	123.5 ± 3.7	116.3 ± 7.4
SGOT, IU/L	66.5 ± 3.8	67.0 ± 5.9	50.8 ± 4.9	80.3 ± 3.5
SGPT, IU/L	30.8 ± 4.1	25.3 ± 3.6	23.0 ± 3.9	32.3 ± 3.9
ALK. PHOS., IU/L	12 ± 1	9 ± 1	12 ± 2	47 ± 31
BUN, MG %	14.0 ± 1.2	15.3 ± .9	12.5 ± 1.9	16.0 ± 2.3
IMMUNOGLOBULIN E, IU/ML	4513 ± 488			3263 ±1006

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 30

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNO FOR 18 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.15 ± .43	7.20 ± .54	7.40 ± .19	7.49 ± .21
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.45 ± .08	.91 ± .15	.88 ± .12	.50 ± .15
HEMATOCRIT, VOL, %	50.0 ± 2.1	49.0 ± 2.0	50.3 ± .9	49.5 ± 1.2
HEMOGLOBIN, GM, %	16.0 ± .6	15.8 ± .6	15.6 ± .1	15.8 ± .5
METHEMOGLOBIN, %	3.2 ± 1.9	.8 ± .8	1.0 ± .3	15.8 ± 5.6[a/]
MCV, CUBIC MICRONS	70.2 ± 1.9	68.7 ± 2.6	67.1 ± 1.4	66.1 ± .7
MCHB, MICRO MICROGMS,	22.5 ± .7	22.1 ± 1.0	20.8 ± .4	20.3 ± .4
MCHBC, GM %	32.0 ± .1	32.2 ± .4	31.0 ± .2	30.7 ± .4[a/]
PLATELETS ($X10^5$ /MM^3)	5.8 ± .3	5.1 ± .3	5.6 ± .6	5.4 ± .6
LEUKOCYTES ($X10^3$ /MM^3)	16.4 ± 1.2	14.4 ± 1.6	17.2 ± .6	16.9 ± 3.2
NEUTROPHILS, %	25.5 ± 2.9	20.8 ± 2.3	24.0 ± 4.9	25.3 ± 6.0
LYMPHOCYTES, %	72.8 ± 2.9	77.8 ± 2.8	73.8 ± 5.3	73.0 ± 5.8
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .5	1.3 ± .5	2.3 ± .5	1.5 ± .6
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 31

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TNG FOR 18 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.49 ± .34	6.62 ± .46	6.95 ± .12	6.81 ± .31
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.73 ± .13 (3)	1.23 ± .44	.78 ± .08	.88 ± .19
HEMATOCRIT, VOL. %	47.0 ± .9	46.3 ± 1.7	46.8 ± 1.1	45.6 ± 2.2
HEMOGLOBIN, GM, %	15.2 ± .3	15.2 ± .7	14.7 ± .1	14.0 ± .7
METHEMOGLOBIN, %	.3 ± .3	1.9 ± 1.5	2.9 ± 1.5	34.5 ± 3.4[a/]
MCV, CUBIC MICRONS	72.8 ± 2.5	70.5 ± 3.3	67.2 ± .8	70.4 ± 1.6
MCHB, MICRO MICROGMS,	23.6 ± .8	23.1 ± .6	21.2 ± .2[a/]	21.5 ± .6
MCH3C, GM %	32.4 ± .4	32.9 ± .7	31.5 ± .6	30.5 ± .4
PLATELETS ($X10^5$ /MM^3)	4.2 ± .2	5.4 ± .2[a/]	5.3 ± .4[a/]	3.5 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	11.2 ± .9	13.1 ± 2.5	11.1 ± .9	10.7 ± 1.0
NEUTROPHILS, %	19.8 ± 3.1	23.0 ± 9.7	30.3 ± 15.6	26.0 ± 1.0
LYMPHOCYTES, %	77.5 ± 2.3	76.0 ± 9.7	67.5 ± 15.2	73.3 ± .9
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
EOSINOPHILS, %	2.3 ± .6	1.0 ± 0.0	2.0 ± .7	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 32

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNG FOR 24 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C. 4)	0.01 (T. 4)	0.1 (T. 4)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.68 ± .47 (3)	6.15 ± .26	6.11 ± .27	5.50 ± .56
HEINZ BODIES. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES. %	1.14 ± .18 (3)	1.02 ± .37	1.78 ± .99	2.49 ± 1.27
HEMATOCRIT. VOL. %	38.7 ± 3.0 (3)	45.0 ± .7	44.0 ± 2.7	39.8 ± 3.4
HEMOGLOBIN. GM. %	13.1 ± .9 (3)	14.6 ± .4	14.3 ± .9	12.0 ± 1.1
METHEMOGLOBIN. %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.9 ± 0.0
MCV. CUBIC MICRONS	68.4 ± 4.0 (3)	73.5 ± 2.8	71.9 ± 1.6	72.9 ± 3.3
MCHB. MICRO MICROGMS.	23.1 ± 1.4 (3)	23.9 ± 1.1	23.4 ± .5	22.0 ± .7
MCHBC. GM %	33.9 ± .4 (3)	32.5 ± .3	32.6 ± .2	30.2 ± .6[a/]
PLATELETS ($X10^5$ /MM^3)	5.2 ± .9 (3)	4.0 ± .6	5.7 ± .4	4.2 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	11.8 ± 1.5 (3)	8.9 ± 1.3	16.0 ± 6.6	16.2 ± 1.7
NEUTROPHILS. %	30.0 ± 5.3 (3)	31.0 ± 2.1	34.8 ± 4.1	44.3 ± 7.2
LYMPHOCYTES. %	65.3 ± 6.1 (3)	68.5 ± 2.3	64.0 ± 3.9	55.3 ± 7.2
BANDS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS. %	1.0 ± 0.6 (3)	.3 ± .3	1.0 ± .7	.3 ± .3
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	3.7 ± 1.9 (3)	.3 ± .3[a/]	.3 ± .3[a/]	0.0 ± 0.0[a/]
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING). MG %	113.3 ± 3.8	104.5 ± 10.9	79.3 ± 6.2[a/]	72.3 ± 3.1[a/]
SGOT. IU/L	57 ± 8	84 ± 12	103 ± 38	222 ± 49[a/]
SGPT. IU/L	24 ± 6	25 ± 7	37 ± 10	126 ± 49[a/]
ALK. PHOS.. IU/L	17 ± 5	19 ± 4	25 ± 7	63 ± 12[a/]
BUN. MG %	27.0 ± 11.0	11.0 ± .4	16.0 ± 1.6	19.8 ± 2.3
IMMUNOGLOBULIN E. IU/ML	563 ± 113			563 ± 113

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 33

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TNG FOR 24 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	6.60 ± .21	5.77 ± .44 (3)	5.26 ± .42	6.71 ± .44
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.38 ± .08	1.07 ± .36 (3)	.93 ± .04	1.64 ± .44[a/]
HEMATOCRIT, VOL, %	40.8 ± 1.1	40.0 ± 2.1 (3)	37.3 ± 2.4	43.0 ± 2.4
HEMOGLOBIN, GM, %	13.8 ± .4	13.2 ± .9 (3)	12.3 ± .7	13.7 ± 1.0
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	61.8 ± .4	69.5 ± 2.1 (3)[a/]	71.1 ± 2.2[a/]	64.2 ± .8
MCMB, MICRO MICROGMS,	20.9 ± .1	23.0 ± .7 (3)	23.6 ± .8	20.4 ± .2
MCHBC, GM %	33.9 ± .2	33.0 ± .5 (3)	33.1 ± .4	31.9 ± .4[a/]
PLATELETS ($\times 10^5$ /MM^3)	5.2 ± .6	4.3 ± .2 (3)	4.8 ± .3	4.4 ± .3
LEUKOCYTES ($\times 10^3$ /MM^3)	4.7 ± .6	9.2 ± 2.3 (3)	4.4 ± .8	6.4 ± 1.0
NEUTROPHILS, %	48.3 ± 6.2	67.7 ± 10.1 (3)	35.8 ± 6.8	43.5 ± 2.6
LYMPHOCYTES, %	49.0 ± 6.2	31.0 ± 10.1 (3)	61.3 ± 6.6	55.5 ± 2.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.0 ± .6	.8 ± .5	2.0 ± 1.4	.5 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .5	.3 ± .3	1.0 ± .6	.5 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	116.0 ± 4.7	101.5 ± 11.7	103.3 ± 1.7	109.8 ± 15.1
SGOT, IU/L	68 ± 7	78 ± 7	128 ± 46	86 ± 4
SGPT, IU/L	39.3 ± 5.7	25.0 ± 5.3	34.5 ± 12.5	36.3 ± 3.9
ALK, PHOS., IU/L	14 ± 3	17 ± 1	14 ± 5	17 ± 6
BUN, MG %	11.3 ± 1.1	13.8 ± 1.7	11.0 ± .6	19.0 ± 2.2[a/]
IMMUNOGLOBULIN E, IU/ML	850 ± 272			663 ± 213

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rate by Dunnett's multiple comparison procedure.

TABLE 34

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNG FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C, 4)	0.01 (T, 3)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.23 ± .32	7.46 ± .17	7.08 ± .31	7.31 ± .28 (3)
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.60 ± .16	.28 ± .09	.40 ± .13	.38 ± .18 (3)
HEMATOCRIT, VOL. %	46.5 ± 1.3	46.3 ± .7	45.0 ± 1.0	45.7 ± .9 (3)
HEMOGLOBIN, GM. %	15.0 ± .4	14.9 ± .6	14.6 ± .5	14.2 ± .4 (3)
METHEMOGLOBIN, %	.4 ± .4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	64.5 ± 1.6	62.1 ± .7	63.7 ± 1.5	62.6 ± 1.4 (3)
MCMB, MICRO MICROGMS.	20.8 ± .6	20.0 ± .5	20.7 ± .4	19.4 ± .4 (3)
MCMBC, GM %	32.3 ± .6	32.2 ± .8	32.5 ± .4	31.0 ± .5 (3)
PLATELETS ($X10^5$ /MM^3)	5.1 ± .6	5.5 ± .4	6.3 ± .4	7.3 ± 1.9 (3)
LEUKOCYTES ($X10^3$ /MM^3)	9.9 ± .4	10.7 ± 1.4	9.2 ± 2.0	14.9 ± 3.2 (3)
NEUTROPHILS, %	25.0 ± 3.2	19.3 ± 2.3	30.5 ± 4.8	25.5 ± 2.3
LYMPHOCYTES, %	74.5 ± 3.0	79.7 ± 2.4	67.8 ± 4.4	73.3 ± 2.6
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	.7 ± .3	.5 ± .3	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	.3 ± .3	1.3 ± .3	1.0 ± .7
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	121.8 ± 5.8	132.7 ± 5.7	137.0 ± 5.0	126.3 ± 6.3
SGOT, IU/L	57.0 ± 3.4	73.3 ± 11.5	81.0 ± 15.8	80.3 ± 9.8
SGPT, IU/L	27.0 ± 1.0	34.0 ± 4.6	51.5 ± 13.1	44.8 ± 9.9
ALK. PHOS., IU/L	48 ± 8	34 ± 7	46 ± 7	36 ± 4
BUN, MG %	12.0 ± 1.2	13.3 ± .3	12.8 ± .3	14.3 ± 1.0
IMMUNOGLOBULIN E, IU/ML	2588 ± 879			1538 ± 302

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 35

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TNG FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C. 4)	0.01 (T. 4)	0.1 (T. 4)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.91 ± .26	5.74 ± .13	6.20 ± .22	5.86 ± .06
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	1.08 ± .39	.78 ± .19	.75 ± .10	1.12 ± .23
HEMATOCRIT, VOL. %	40.8 ± 1.4	40.5 ± 1.0	41.8 ± .6	39.5 ± 1.0
HEMOGLOBIN, GM. %	13.1 ± .5	13.1 ± .2	13.3 ± .2	12.6 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	69.3 ± 3.1	70.6 ± .9	67.5 ± 1.7	67.3 ± 1.3
MCH, MICRO MICROGMS.	22.3 ± .9	22.9 ± .1	21.5 ± .7	21.5 ± .7
MCHBC, GM %	32.2 ± .5	32.5 ± .4	31.9 ± .3	31.9 ± .4
PLATELETS ($X10^5$ /MM^3)	4.5 ± .7	5.7 ± .2	5.4 ± .2	7.9 ± 1.6
LEUKOCYTES ($X10^3$ /MM^3)	5.9 ± .8	4.8 ± .4	5.1 ± .3	6.5 ± .9
NEUTROPHILS, %	23.3 ± 3.3	28.3 ± 4.5	24.3 ± 4.6	29.3 ± 3.0
LYMPHOCYTES, %	75.3 ± 2.9	71.0 ± 4.8	75.0 ± 4.7	69.0 ± 3.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .3	.5 ± .3	.5 ± .3	1.3 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .6	.3 ± .3	.3 ± .3	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	122.5 ± 6.2	128.0 ± 3.9	129.8 ± 5.5	121.3 ± 8.1
SGOT, IU/L	85.8 ± 13.0	63.3 ± 3.1	64.8 ± 12.5	72.5 ± 9.6
SGPT, IU/L	31.3 ± 5.2	27.8 ± 1.4	27.0 ± 2.1	32.3 ± 3.9
ALK. PHOS., IU/L	23 ± 9	16 ± 3	13 ± 3	14 ± 2
BUN, MG %	14.0 ± .7	11.8 ± 1.1	13.3 ± .8	12.3 ± .6
IMMUNOGLOBULIN E, IU/ML	2500 ± 513			917 ± 280 (3)

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rate by Dunnett's multiple comparison procedure.

TABLE 36

LABORATORY DATA OF MALE RATS AFTER FEEDING OF TNG FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C. 3)	0.01 (T. 1)	0.1 (T. 3)	1 (T. 3)
ERYTHROCYTES ($\times 10^6$ /MM^3)	4.94 ± 1.33		4.74 ± .54	5.51 ± .29
HEINZ BODIES, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	5.30 ± 3.89	.56	2.31 ± .53	2.70 ± .93
HEMATOCRIT, VOL. %	36.3 ± 6.3		37.7 ± .7	38.7 ± 2.0
HEMOGLOBIN, GM. %	11.8 ± 2.4		11.6 ± 1.3	12.2 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	79.2 ± 10.6		81.5 ± 9.3	70.2 ± 1.6
MCHB, MICRO MICROGMS.	25.1 ± 2.3		24.4 ± .1	22.1 ± .1
MCHBC, GM %	32.0 ± 1.3		30.7 ± 3.2	31.6 ± .8
PLATELETS ($\times 10^5$ /MM^3)	2.6 ± .5		5.7 ± .2 a/	4.2 ± .5
LEUKOCYTES ($\times 10^3$ /MM^3)	6.5 ± 1.7		12.8 ± 2.8	13.1 ± 3.7
NEUTROPHILS, %	31.3 ± 3.3	18.0	28.0 ± 3.6	57.7 ± 13.4
LYMPHOCYTES, %	68.3 ± 3.0	80.0	71.3 ± 3.2	42.0 ± 13.3
BANDS, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	2.0	.7 ± .7	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	28.7 ± 28.7	0.0	0.0 ± 0.0	1.3 ± 1.3
GLUCOSE (FASTING), MG %	98.7 ± 10.9	123.0	97.7 ± 16.9	74.3 ± 28.4
SGOT, IU/L	51 ± 6	59	61 ± 1	661 ± 721
SGPT, IU/L	20 ± 1	21	21 ± 3	498 ± 417
ALK. PHOS., IU/L	104 ± 70	27	57 ± 17	104 ± 56
BUN, MG %	51.0 ± 18.1	16.0	37.7 ± 20.7	38.3 ± 18.9
IMMUNOGLOBULIN E, IU/ML	3117 ±1097			600 ± 150

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 37

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF TNT FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: % in feed	0 (C. 3)	0.01 (T. 4)	0.1 (T. 3)	1 (T. 3)
ERYTHROCYTES ($X10^6$ /MM^3)	6.23 ± .22	5.49 ± .32	5.07 ± .29	5.07 ± .36
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.95 ± .15	1.11 ± .43	1.47 ± .30	2.17 ± .44
HEMATOCRIT, VOL. %	43.3 ± .3	37.0 ± .7[a/]	36.0 ± 1.5[a/]	36.0 ± 2.5[a/]
HEMOGLOBIN, GM. %	14.6 ± .2	12.8 ± .4	12.2 ± .6[a/]	11.2 ± .9[a/]
METHEMOGLOBIN, %	0.0 ± 0.0	.4 ± .4	0.0 ± 0.0	.8 ± .8
MCV, CUBIC MICRONS	69.7 ± 2.7	67.8 ± 2.9	71.1 ± 1.3	71.0 ± 1.4
MCHB, MICRO MICROGMS.	23.5 ± .8	23.5 ± .6	24.0 ± .5	22.1 ± .5
MCHBC, GM %	33.7 ± .1	34.6 ± .5	33.8 ± .4	31.1 ± .3[a/]
PLATELETS ($X10^5$ /MM^3)	4.3 ± .4	4.5 ± .5	4.8 ± .2	4.4 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	5.1 ± .7	8.8 ± 4.1	7.7 ± .8	7.8 ± 1.5
NEUTROPHILS, %	26.7 ± 2.3	29.0 ± 5.9	30.7 ± 6.2	48.3 ± 8.4
LYMPHOCYTES, %	70.0 ± 3.8	69.0 ± 6.1	64.3 ± 5.9	51.3 ± 8.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	3.3 ± 1.7	.5 ± .3	3.0 ± 0.0	0.0 ± 0.0[a/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	1.3 ± .6	2.0 ± 1.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.7 ± .7
GLUCOSE (FASTING), MG %	113.7 ± 19.2	87.3 ± 10.6	111.3 ± 16.3	92.0 ± 4.2
SGOT, IU/L	65 ± 7	145 ± 59	63 ± 7	122 ± 23
SGPT, IU/L	28.7 ± 4.4	32.3 ± 10.8	20.0 ± 2.0	57.7 ± 11.9
ALK. PHOS., IU/L	22 ± 4	54 ± 14	53 ± 7	41 ± 8
BUN, MG %	14.3 ± 1.9	12.5 ± .5	13.7 ± 1.2	23.3 ± 5.8
IMMUNOGLOBULIN E, IU/ML	3517 ±1025			2067 ± 651

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 38

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED TNG FOR 12 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	781 ± 32[a]	2.11 ± 0.01	1.83 ± 0.09	18.5 ± 1.2	3.8 ± 0.3	1.04 ± 0.10	3.9 ± 0.2	
	0.01	811 ± 29[b]	2.20 ± 0.06	1.71 ± 0.04	18.2 ± 0.8	3.8 ± 0.0	0.92 ± 0.02	3.9 ± 0.1	
	0.1	812 ± 38[b]	2.29 ± 0.11	1.96 ± 0.07	21.9 ± 1.0	4.4 ± 0.3	0.99 ± 0.10	3.6 ± 0.1	
	1	661 ± 34[a]	2.22 ± 0.05	2.20 ± 0.46	28.3 ± 3.0[c]	4.6 ± 0.2	1.13 ± 0.05	3.5 ± 0.2	
Female	0	376 ± 24[a]	1.88 ± 0.04	1.30 ± 0.04	10.5 ± 0.7	2.2 ± 0.1	0.57 ± 0.04		0.123 ± 0.008
	0.01	410 ± 13[a]	1.97 ± 0.03	1.23 ± 0.04	10.9 ± 0.2	2.4 ± 0.0	0.56 ± 0.02		0.123 ± 0.013
	0.1	464 ± 29[a,c]	1.89 ± 0.05	1.20 ± 0.05	12.5 ± 0.9	2.5 ± 0.1	0.61 ± 0.06		0.136 ± 0.017
	1	305 ± 13[a]	1.87 ± 0.05	1.24 ± 0.12	15.7 ± 1.9[c]	2.7 ± 0.2[c]	0.67 ± 0.07		0.160 ± 0.016

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.27 ± 0.01	0.24 ± 0.01	2.36 ± 0.09	0.49 ± 0.04	0.132 ± 0.008	0.50 ± 0.02	
	0.01	0.27 ± 0.01	0.21 ± 0.01	2.25 ± 0.04	0.47 ± 0.02	0.114 ± 0.005	0.48 ± 0.02	
	0.1	0.28 ± 0.02	0.24 ± 0.02	2.70 ± 0.03	0.55 ± 0.02	0.121 ± 0.010	0.45 ± 0.02	
	1	0.34 ± 0.02[c]	0.33 ± 0.05	4.26 ± 0.34[c]	0.71 ± 0.04[c]	0.173 ± 0.014[c]	0.53 ± 0.04	
Female	0	0.50 ± 0.03	0.35 ± 0.02	2.80 ± 0.19	0.58 ± 0.03	0.153 ± 0.013		0.033 ± 0.003
	0.01	0.48 ± 0.01	0.30 ± 0.02	2.68 ± 0.15	0.59 ± 0.01	0.137 ± 0.008		0.030 ± 0.003
	0.1	0.41 ± 0.02[c]	0.26 ± 0.01	2.72 ± 0.23	0.54 ± 0.05	0.133 ± 0.013		0.029 ± 0.002
	1	0.61 ± 0.01[c]	0.41 ± 0.04	5.07 ± 0.72[c]	0.89 ± 0.05[c]	0.219 ± 0.014[c]		0.052 ± 0.004[c]

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					
		Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.87 ± 0.04	8.79 ± 0.60	1.83 ± 0.16	0.494 ± 0.047	1.84 ± 0.08	
	0.01	0.78 ± 0.04	8.29 ± 0.43	1.73 ± 0.05	0.419 ± 0.005	1.77 ± 0.08	
	0.1	0.86 ± 0.01	9.64 ± 0.77	1.95 ± 0.18	0.436 ± 0.062	1.58 ± 0.05	
	1	1.01 ± 0.23	12.85 ± 1.67	2.09 ± 0.07	0.509 ± 0.020	1.56 ± 0.07[c]	
Female	0	0.59 ± 0.03	5.59 ± 0.37	1.15 ± 0.04	0.303 ± 0.022		0.065 ± 0.003
	0.01	0.62 ± 0.03	5.56 ± 0.19	1.22 ± 0.01	0.285 ± 0.011		0.062 ± 0.006
	0.1	0.64 ± 0.02	6.61 ± 0.40	1.32 ± 0.06	0.323 ± 0.024		0.071 ± 0.007
	1	0.66 ± 0.07	8.31 ± 1.13[c]	1.46 ± 0.09[c]	0.358 ± 0.02		0.085 ± 0.007

a/ Mean ± standard error of four rats.
b/ Mean ± standard error of three rats.
c/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 39

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED TNG FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% of feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	788 ± 17[a]	2.21 ± 0.06	2.07 ± 0.16	17.5 ± 0.5	4.0 ± 0.2	0.95 ± 0.05	3.7 ± 0.1	
	0.01	805 ± 37[b]	2.16 ± 0.02	1.96 ± 0.14	17.2 ± 0.4	3.7 ± 0.1	0.85 ± 0.08	3.7 ± 0.2	
	0.1	755 ± 48[a]	2.12 ± 0.04	1.90 ± 0.03	17.7 ± 1.7	3.5 ± 0.2	0.88 ± 0.02	4.0 ± 0.2	
	1	611 ± 38[a,c]	2.16 ± 0.11	1.78 ± 0.19	24.8 ± 1.0[c]	4.1 ± 0.4	1.25 ± 0.09[c]	3.9 ± 0.3	
Female	0	454 ± 30[a]	1.91 ± 0.02	1.31 ± 0.03	9.2 ± 2.1	2.4 ± 0.2	0.73 ± 0.18		0.121 ± 0.013
	0.01	465 ± 27[a]	1.68 ± 0.22	1.35 ± 0.09	10.7 ± 0.6	2.3 ± 0.1	0.66 ± 0.06		0.183 ± 0.041
	0.1	436 ± 24[a]	1.52 ± 0.15	1.31 ± 0.12	11.7 ± 0.8	2.2 ± 0.1	0.62 ± 0.03		0.387 ± 0.161
	1	324 ± 24[a,c]	1.90 ± 0.06	1.14 ± 0.08	14.0 ± 1.4[c]	2.4 ± 0.2	0.69 ± 0.06		0.171 ± 0.010

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.28 ± 0.01	0.26 ± 0.03	2.22 ± 0.02	0.51 ± 0.03	0.121 ± 0.007	0.47 ± 0.01	
	0.01	0.27 ± 0.01	0.24 ± 0.01	2.14 ± 0.07	0.47 ± 0.03	0.108 ± 0.016	0.45 ± 0.02	
	0.1	0.28 ± 0.02	0.25 ± 0.02	2.41 ± 0.39	0.47 ± 0.06	0.118 ± 0.10	0.54 ± 0.03	
	1	0.36 ± 0.04	0.30 ± 0.05	4.10 ± 0.31[c]	0.69 ± 0.10	0.207 ± 0.024[c]	0.66 ± 0.09	
Female	0	0.43 ± 0.03	0.29 ± 0.02	1.76 ± 0.41	0.53 ± 0.05	0.167 ± 0.047		0.027 ± 0.003
	0.01	0.36 ± 0.05	0.29 ± 0.03	2.33 ± 0.21	0.50 ± 0.04	0.144 ± 0.018		0.041 ± 0.041
	0.1	0.35 ± 0.03	0.31 ± 0.04	2.72 ± 0.33	0.52 ± 0.05	0.144 ± 0.014		0.089 ± 0.036
	1	0.60 ± 0.04[c]	0.36 ± 0.05	4.46 ± 0.68[c]	0.77 ± 0.11	0.219 ± 0.031		0.053 ± 0.002

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					
		Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.94 ± 0.07	7.98 ± 0.43	1.84 ± 0.11	0.432 ± 0.023	1.69 ± 0.07	
	0.01	0.91 ± 0.06	7.95 ± 0.21	1.73 ± 0.06	0.395 ± 0.044	1.69 ± 0.09	
	0.1	0.90 ± 0.03	8.36 ± 0.78	1.64 ± 0.10	0.416 ± 0.004	1.91 ± 0.13	
	1	0.82 ± 0.07	11.57 ± 0.82[c]	1.90 ± 0.16	0.580 ± 0.046[c]	1.82 ± 0.09	
Female	0	0.68 ± 0.02	4.24 ± 1.08	1.26 ± 0.11	0.385 ± 0.099		0.063 ± 0.006
	0.01	0.89 ± 0.22	7.04 ± 1.70	1.49 ± 0.31	0.429 ± 0.104		0.130 ± 0.055
	0.1	0.93 ± 0.21	8.16 ± 1.62	1.55 ± 0.24	0.424 ± 0.062		0.262 ± 0.100
	1	0.60 ± 0.06	7.38 ± 0.74	1.26 ± 0.12	0.363 ± 0.038		0.090 ± 0.005

a/ Mean ± standard error of four rats.

b/ Mean ± standard error of three rats.

c/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 40

SUMMARY OF LESIONS IN MALE RATS FED TNG FOR 12 MONTHS

Dose (% in feed):	0				0.01			0.1			1			
Rat No.:	301	302	303	304	310	311	312	317	319	320	325	326	327	328
Treatment-Related Lesions[a/]														
Liver														
Cholangiofibrosis												±		2
Foci or areas of hepatocellular alteration					1		1		2	2	2			4
Neoplastic nodules												4		
Hepatocellular carcinoma												X		
Spleen														
Excessive pigmentation									1		1	2	2	2
Kidney														
Excessive pigmentation													1	1
Other Lesions														
Pituitary Gland														
Chromophobe adenoma								X						
Colloid cyst		1												
Adrenal Gland														
Cystic degeneration	1							2						1
Focal fatty change	1												1	
Thyroid														
Squamous metaplastic follicle												1		
Thyroiditis											1			
Trachea														
Tracheitis		1		3				1	1	3				1
Lung														
Peribronchiolar cuffings (CMP)	2	1	2	2	1	1	1	1	1	1	1		1	1
Abscess									1					
Heart failure cells													2	
Heart														
Focal myocarditis	1	1	1	1	1	2		1	1		1			1
Myocardial fibrosis						1								
Valvular insufficiency with cardiac dilatation													2	
Liver														
Bile duct proliferation				2	1	1	1		1	1				
Portal inflammation	1	1					1		1		1			
Focal necrosis				1	1								3	
Epididymis														
Interstitial inflammation	1	1												
Epithelial vacuolization									1					1
Prostate														
Hypertrophy			1	1										
Pancreas														
Acinar atrophy	1		1				1		1				1	1
Islet fibrosis				1			1			1				
Lymph Node														
Hemosiderosis					1		1	1			1			
Hemorrhage													2	
Intestine														
Parasitism						1	1	1						
Kidney														
Focal tubular nephrosis		1			1		1		1	1	1	1		
Pyelitis	1													
Perivascular cuffings	1	1			1					1		1	1	1
Chronic progressive nephrosis				3				2						
Eye														
Synechia	1													
Meibomian gland hyperplasia	1													
Bone marrow														
M/E ratio	1.0	1.3	1.0	1.1	1.2	1.3	1.1	1.0	1.0	1.1	1.5	1.4	1.1	1.0

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 41

SUMMARY OF LESIONS IN FEMALE RATS FED TNG FOR 12 MONTHS

Dose (% in feed):	0				0.01				0.1				1			
Rat No.:	351	352	353	354	359	360	361	362	367	368	369	370	375	376	377	378
Treatment-Related Lesions[a/]																
Liver																
Cholangiofibrosis													1	2	1	2
Foci or areas of hepatocellular alteration										2	2	2	4	4	4	4
Spleen																
Excessive pigmentation														1	1	1
Kidney																
Excessive pigmentation																
Other Lesions																
Pituitary Gland																
Chromophobe adenoma							X									
Colloid cyst	1															
Adrenal Gland																
Cystic degeneration	2	1	1		1	1	1			1		1				
Thyroid																
Squamous metaplastic follicle					1											
Thyroiditis														1		
Trachea																
Tracheitis				1				1					1		1	1
Lung																
Peribronchiolar cuffings (CMP)	2	1	1	1	1	1	1	3	1	1	1	1	1	2	1	1
Heart																
Focal myocarditis	1							1			1	1		1	1	1
Liver																
Bild duct proliferation						1		1		1	1	1				
Portal inflammation		1														
Epididymis																
Epithelial vacuolization														3		
Ovary																
Ovarian cyst				1									±			
Uterus																
Endometritis							1									1
Pancreas																
Acinor atrophy	1	1					1									
Islet fibrosis				1						1						
Intestine																
Lymphoid hyperplasia					1											
Eosinophilic granuloma			X													
Kidney																
Focal Tubular nephrosis												1				
Microscopic calculi	1		1		1			1	1	1		1				
Pyelitis							1									
Perivascular cuffings				1	1		1			1				1	1	1
Skin																
Squamous cell carcinoma												±				
Bone Marrow																
M/E ratio	0.8	1.3	1.0	1.4	0.9	0.9	1.1	1.1	1.5	1.5	1.3	1.4	1.7	1.4		1.5

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 42

SUMMARY OF LESIONS IN MALE RATS FED TNG FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0				0.01			0.1				1			
Rat No.:	305	306	307	308	313	315	316	321	322	323	324	329	330	331	332
Treatment-Related Lesions[a/]															
Liver															
Cholangiofibrosis												4	3	4	4
Areas or foci of hepatocellular alteration			1			1	1	1	2	1	1	1	2	3	2
Neoplastic nodules									1			1	2		
Spleen															
Excessive pigmentation					1		1					1	3	3	3
Kidney															
Excessive pigmentation															1
Other Lesions															
Adrenal Gland															
Cystic degeneration						1									
Thyroid															
Metaplastic follicle	1			1											
Trachea															
Tracheitis	1	1	1	1			1		1	2	3	1			1
Heart															
Focal myocarditis		1	1	1			1	1						1	
Lung															
Chronic murine pneumonia	1	3		1	2	1	1	2	1	1	1	1	1	1	1
Liver															
Bile duct hyperplasia	1	2	1	1		1	1			1	1				
Granuloma											X				
Liver necrosis					1			1							
Portal inflammation			1	1					1						
Fatty change										1					
Testis															
Interstitial cell tumor												X			
Epididymis															
Epithelial vacuolization		1				1		1				1	1		1
Pancreas															
Focal acinar atrophy	1				1	1	1						1		1
Stomach															
Calcification		1													
Intestine															
Parasitism	1												1	1	
Lymphoid hyperplasia	1														
Kidney															
Perivascular cuffs			1	1	1	1	1	1		1				1	
Chronic progressive nephrosis		2							2						
Tubular nephrosis			1		1		1								
Cyst											1				
Skin															
Epidermal inclusion cyst										3					
Eye															
Calcification of cornea						1									
Brain															
Astrocytoma								X							
Bone Marrow Smear															
M/E ratio	1.9	0.9	1.3	1.3	1.0	0.9	1.0	0.9	1.0	1.3	1.1	1.5	1.8	1.4	1.3

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable. X = present.

TABLE 43

SUMMARY OF LESIONS IN FEMALE RATS FED TNG FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0				0.01				0.1				1			
Rat No.:	355	356	357	358	363	364	365	366	371	372	373	374	379	380	381	382
Treatment-Related Lesions[a]																
Liver																
Cholangiofibrosis													4	4	3	4
Areas or foci of hepatocellular alteration		1				1	1			1			2	2	2	3
Neoplastic nodules																2
Spleen																
Excessive pigmentation									1		1		3	3	3	3
Kidney																
Excessive pigmentation													1	1	1	2
Other Lesions																
Pituitary																
Chromophobe adenoma			X													
Adrenal Gland																
Cystic degeneration		2	2	2	1	1	1	3	2	1	2	1				
Thyroid																
Metaplastic follicle			1													
Trachea																
Tracheitis		1			2			1								
Heart																
Focal myocarditis				1		1		1			1	1			1	
Lung																
Chronic murine pneumonia	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Focal emphysema					1											
Lipoid granuloma									X							
Liver																
Bile duct hyperplasia	1		1			1	1		1	1	1					
Liver necrosis						1					1					
Portal inflammation	1	1	1						1		1					
Fatty change			2								2					
Spleen																
Extramedullary hematopoiesis			1													
Ovary																
Ovarian cyst							1				1				1	
Uterus																
Endometritis			1		1											
Pancreas																
Focal acinar atrophy	1															
Focal pancreatitis						1										
Salivary Gland																
Focal mononuclear cell infiltration											1					
Stomach																
Etopic pancreatic tissue									1							
Calcification					1											
Intestine																
Parasitism				1												
Lymphoid hyperplasia	1					1					1			1		
Kidney																
Perivascular cuffs	1									1	1					
Tubular nephrosis			1						1							1
Microscopic calcinosis		1		1	1	1	1	1		1						
Peripyelitis		1		2												1
Adenoma			X													
Urinary Bladder																
Mononuclear cell foci														1		
Omentum																
Foreign body granuloma			X													
Eye																
Calcification of periocular muscle									1							
Brain																
Calcification									2							
Bone Marrow Smear																
M/E Ratio	1.0	1.0	1.3	1.4	0.9	1.0	1.2	1.1	0.9	1.5	1.2	1.0	1.2	0.8		1.5

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 44

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED TNG FOR 24 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	726 ± 48[a/]	2.22 ± 0.13	2.15 ± 0.28	20.6 ± 2.8	6.6 ± 1.2	1.35 ± 0.18	3.67 ± 0.21	
	0.01	813 ± 60[a/]	2.23 ± 0.04	1.83 ± 0.13	16.0 ± 1.1	4.1 ± 0.2	0.99 ± 0.05	3.98 ± 0.76	
	0.1	713 ± 33[b/]	2.18 ± 0.03	2.03 ± 0.21	24.2 ± 2.7	5.8 ± 0.6	1.17 ± 0.18	4.20 ± 0.53	
	1	557 ± 25[c,f/]	2.21 ± 0.04	1.83 ± 0.08	74.0 ± 16.6[b/]	5.6 ± 0.3	1.79 ± 0.25	4.62 ± 0.79	
Female	0	536 ± 24[b/]	1.96 ± 0.03	1.49 ± 0.05	14.9 ± 1.5	3.0 ± 0.2	1.15 ± 0.43		0.213 ± 0.029
	0.01	515 ± 37[d/]	1.95 ± 0.01	1.39 ± 0.06	13.9 ± 0.3	3.1 ± 0.1	1.47 ± 0.75		0.587 ± 0.385
	0.1	444 ± 14[d,f/]	1.95 ± 0.06	1.37 ± 0.04	14.6 ± 0.9	2.9 ± 0.2	0.[illegible] ± 0.04		0.200 ± 0.038
	1	305 ± 6[e,f/]	1.90 ± 0.02	1.18 ± 0.04[f/]	31.3 ± 2.[illegible][f/]	3.3 ± 0.1	0.91 ± 0.03		0.599 ± 0.248

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.31 ± 0.01	0.30 ± 0.03	2.82 ± 0.28	0.91 ± 0.15	0.188 ± 0.027	0.51 ± 0.03	
	0.01	0.27 ± 0.03	0.22 ± 0.04	1.90 ± 0.05	0.50 ± 0.03	0.118 ± 0.009	0.47 ± 0.08	
	0.1	0.31 ± 0.02	0.29 ± 0.03	3.45 ± 0.42	0.83 ± 0.10	0.167 ± 0.028	0.62 ± 0.11	
	1	0.40 ± 0.02[f/]	0.33 ± 0.02	13.29 ± 2.76[f/]	1.62 ± 0.05	0.319 ± 0.041	0.85 ± 0.16	
Female	0	0.37 ± 0.02	0.28 ± 0.01	2.74 ± 0.19	0.56 ± 0.03	0.207 ± 0.072		0.041 ± 0.[illegible]
	0.01	0.39 ± 0.03	0.28 ± 0.01	2.76 ± 0.17	0.61 ± 0.03	0.326 ± 0.179		0.128 ± 0.090
	0.1	0.44 ± 0.02	0.31 ± 0.01	3.28 ± 0.16	0.66 ± 0.04	0.158 ± 0.006		0.046 ± 0.009
	1	0.63 ± 0.02[f/]	0.39 ± 0.01[f/]	10.37 ± 0.73[f/]	1.08 ± 0.03[f/]	0.300 ± 0.010	0	0.189 ± 0.075

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					
		Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.97 ± 0.13	9.23 ± 0.9[illegible]	2.98 ± 0.52	0.[illegible]5 ± 0.093	1.67 ± 0.13	
	0.01	0.82 ± 0.06	7.21 ± 0.49	1.87 ± 0.13	0.444 ± 0.020	1.77 ± 0.30	
	0.1	0.94 ± 0.11	11.12 ± 1.25	2.67 ± 0.30	0.536 ± 0.084	1.92 ± 0.23	
	1	0.83 ± 0.03	33.82 ± 7.52[f/]	2.55 ± 0.12	0.809 ± 0.108	2.09 ± 0.35	
Female	0	0.76 ± 0.03	7.59 ± 0.76	1.53 ± 0.10	0.577 ± 0.208		0.109 ± 0.015
	0.01	0.72 ± 0.03	7.13 ± 0.19	1.59 ± 0.05	0.750 ± 0.376		0.305 ± 0.202
	0.1	0.70 ± 0.02	7.46 ± 0.34	1.49 ± 0.07	0.362 ± 0.027		0.103 ± 0.020
	1	0.63 ± 0.02[f/]	16.48 ± 1.02[f/]	1.74 ± 0.07	0.481 ± 0.015		0.318 ± 0.132

a/ Mean ± standard error of four rats.
b/ Mean ± standard error of nine rats.
c/ Mean ± standard error of eight rats.
d/ Mean ± standard error of seven rats.
e/ Mean ± standard error of 18 rats.
f/ Significantly different from control rats by Dunnett's multiple comparison procedure.

TABLE 45

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED TNG FOR 24 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	604 ± 46[a]	2.20 ± 0.05	2.50 ± 0.22	20.0 ± 2.1	7.5 ± 1.1	1.38 ± 0.44	3.13 ± 0.65	
	0.1	730 ± 27[b]	2.27 ± 0.02	2.23 ± 0.35	22.2 ± 2.2	5.8 ± 1.3	1.24 ± 0.08	3.32 ± 0.32	
	1	533 ± 89[a]	2.15 ± 0.12	1.73 ± 0.31	57.1 ± 12.4[c]	4.8 ± 0.4	1.27 ± 0.53	3.12 ± 0.99	
Female	0	477 ± 28[a]	1.89 ± 0.02	1.64 ± 0.04	13.0 ± 1.2	3.0 ± 0.2	0.76 ± 0.13		0.860 ± 0.586
	0.01	459 ± 62[b]	1.92 ± 0.02	1.57 ± 0.09	13.8 ± 3.0	2.7 ± 0.1	2.20 ± 1.63		0.183 ± 0.032
	0.1	518 ± 49[a]	1.96 ± 0.05	1.39 ± 0.12	13.9 ± 2.4	2.6 ± 0.2	0.69 ± 0.06		0.150 ± 0.027
	1	316 ± 8[a]	1.92 ± 0.10	1.42 ± 0.04	37.3 ± 9.8[c]	3.1 ± 0.2	0.99 ± 0.15		0.180 ± 0.050

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.37 ± 0.02	0.42 ± 0.07	3.34 ± 0.37	1.27 ± 0.26	0.24 ± 0.10	0.51 ± 0.08	
	0.1	0.31 ± 0.01	0.31 ± 0.05	3.05 ± 0.35	0.81 ± 0.20	0.17 ± 0.02	0.46 ± 0.05	
	1	0.43 ± 0.08	0.32 ± 0.01	11.86 ± 4.00[c]	0.93 ± 0.10	0.22 ± 0.07	0.55 ± 0.12	
Female	0	0.40 ± 0.03	0.35 ± 0.01	2.72 ± 0.24	0.63 ± 0.03	0.159 ± 0.024		0.196 ± 0.140
	0.01	0.44 ± 0.05	0.35 ± 0.02	2.94 ± 0.24	0.62 ± 0.06	0.383 ± 0.240		0.042 ± 0.010
	0.1	0.39 ± 0.05	0.27 ± 0.00	2.65 ± 0.27	0.51 ± 0.03	0.137 ± 0.022		0.030 ± 0.007
	1	0.61 ± 0.03[c]	0.45 ± 0.02[d]	11.88 ± 3.14[c]	0.99 ± 0.04[c]	0.316 ± 0.052		0.057 ± 0.015

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					
		Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	1.14 ± 0.13	9.12 ± 1.01	3.42 ± 0.59	0.64 ± 0.22	1.41 ± 0.27	
	0.1	0.98 ± 0.15	9.77 ± 1.00	2.57 ± 0.56	0.54 ± 0.04	1.46 ± 0.13	
	1	0.81 ± 0.14	26.19 ± 4.84[c]	2.23 ± 0.17	0.58 ± 0.22	1.48 ± 0.51	
Female	0	0.87 ± 0.03	6.85 ± 0.71	1.58 ± 0.12	0.40 ± 0.07		0.449 ± 0.305
	0.01	0.81 ± 0.05	7.18 ± 1.54	1.43 ± 0.04	1.14 ± 0.85		0.095 ± 0.016
	0.1	0.71 ± 0.08	7.18 ± 1.37	1.35 ± 0.11	0.35 ± 0.02		0.077 ± 0.013
	1	0.74 ± 0.05	20.02 ± 5.85[c]	1.63 ± 0.14	0.53 ± 0.10		0.092 ± 0.021

a/ Mean ± standard error of three rats.
b/ Mean ± standard error of four rats.
c/ Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 44

SUMMARY OF LESIONS OF MALE RATS FED 0%, 0.01% THS or 0.1% THS FOR 24 MONTHS

Dose (% in food):	0				0.01				0.1							
Rat No.:	009	012	021	023	101	104	105	108	131	134	135	137	138	141	142	143	148
Treatment-Related Lesions[a]																	
Pituitary																	
Chromophobe adenoma			X	X						X		X	X	X			X
Liver																	
Areas or foci of hepatocellular alteration			1		1			1	1	1	1		1	2	1		
Hepatocellular carcinoma															X		
Testis																	
Interstitial cell tumor				X	X				X		X						
Other Lesions																	
Adrenal Gland																	
Cystic degeneration		1		1												1	
Cortical adenoma			X														
Pheochromocytoma	X						X					X					
Extramedullary cortical nodule											X	X					
Thyroid																	
C cell hyperplasia	1	1					1							1			
Follicular epithelium adenoma											X						
Hyperplasia of parathyroid		1															1
Trachea																	
Tracheitis	1				1		1	1			1			1	1		
Lung																	
Chronic murine pneumonia		2		2		1	1	1	1	1	3		1	1	1	2	1
Lymphoreticular tumor												X					
Heart																	
Myocardial degeneration/fibrosis	1	2	1			1	1	1	1	1		1		1	1	1	
Auricular thrombus													4				
Liver																	
Simple bile duct hyperplasia	1	1		1	1		1	1	2	1	1			1		1	1
Portal inflammation		1	1	1			1			1			1	1		1	1
Cystic degeneration						1	1								1	1	1
Focal hepatocellular degeneration				2									1				
Microgranuloma			1														
Lymphoreticular tumor												X					
Spleen																	
Extramedullary hematopoiesis												1					
R. E. cell hyperplasia												2					
Testis																	
Atrophy and degeneration of seminiferous tubules						3			2					3			
Periarteritis nodosa		A															1
Epididymis																	
Epithelial vacuolization	1	A	1								1						
Prostate		O															
Prostatitis						1			1	1							
Lymphoreticular tumor												X					
Seminal Vesicle		O															
Atrophy									1								
Lymphoreticular tumor												X					
Pancreas																	
Focal acinar atrophy						1									1		
Foci of eosinophilic cells														2			
Lymph Node																	
Lymphoreticular tumor												X					
Lymphoid hyperplasia										1							
Hemangioma							X										
Stomach																	
Dilated crypts							1					1					
Intestine																	
Parasitism		1	1	1	1												
Calcification			1								1						
Lymphoreticular tumor												X					
Kidney																	
Chronic senile nephropathy	3	4	1	3	1	2		1	2	4	1	1	1	2	1	1	3
Lymphoreticular tumor												X					
Cyst												1					
Foci of eosinophilic cells							1	1									
Urinary Bladder																	
Foci of eosinophilic cells					1												
Rib																	
Hypocellularity of bone marrow								1		2							
Eye																	
Keratitis										1							
Skeletal Muscle																	
Focal degeneration/myositis							1										

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 47

SUMMARY OF LESIONS OF FEMALE RATS FED 0%, 0.01% TRQ OR 0.1% TRQ FOR 24 MONTHS

Dose (% in feed):	0									0.01							0.1						
Rat No.:	033	034	036	038	040	041	043	045	047	201	203	205	206	207	209	214	231	233	236	238	240	241	242
Treatment-Related Lesions[a/]																							
Pituitary																							
Chromophobe adenoma				X		X	X	X	X	X	X		X		X	X	X	X	X			X	X
Liver																							
Areas or foci of hepatocellular alteration						1	1			1			1				2	2	2	2	1		1
Neoplastic nodules																			X				
Hepatocellular carcinoma																	X						
Mammary Gland																							
Fibroadenoma	X	X	X						X		X		X			X						X	X
Adenoma		X							X									X		X	X		
Adenocarcinoma-carcinoma										X												X	
Other Lesions																							
Adrenal Gland																							
Cystic degeneration	2	3	3	2	3	1		2	1	1	2	3	3	3	3	1	1	2	1		2	3	2
Pheochromocytoma										X			X										
Extraregular cortical nodule		X	X																				
Thyroid																							
C cell hyperplasia									1					1				1		1			
C cell adenoma			X													X							
Squamous metaplastic follicle								1															
Trachea																							
Tracheitis								1													1		1
Lungs																							
Chronic murine pneumonia	1	1	1	1	1	1	1	1	1	1	1	1	1	1			1	1			1	1	
Heart																							
Myocardial degeneration/fibrosis	1		1	1		1				1	1			1							1		
Liver																							
Simple bile duct hyperplasia		2		1				2						1	3	1	1	1	1	1	1	2	
Portal inflammation				1			1		1				1				1						
Telangiectasis																					2		1
Cystic degeneration																							1
Focal hepatocellular degeneration	1	1		2	1		2	1	3	1	1			1									
Microgranuloma			1														1						
Lymphoreticular tumor												X			X								
Spleen																							
Extramedullary hematopoiesis									1			1											
R. E. cell hyperplasia							4	1															
Lymphoreticular tumor															X								
Ovary																							
Ovarian cyst						X				X			X		X				X				
Lymphoreticular tumor												X											
Uterus																							
Endometritis						1	4				1		2			1		1					2
Pancreas																							
Islet cells hyperplasia						1	1							1									
Lymph Node																							
Lymphoreticular tumor												X											
Lymphoid hyperplasia						1		1									1						
Stomach																							
Dilated crypts			1						1				1	1								1	
Submucosal edema															2								
Peritonitis												2											
Intestine																							
Parasitism			1								1			1					1			1	
Lymphoreticular tumor												X											
Kidney																							
Microcalculi				1			1				1	1	1		1						1	1	1
Tubular nephrosis							1					2										3	
Foci of mononuclear cells				1													1			1	1	1	1
Skin																							
Papilloma (epithelial tumor)								X															
Abdominal Cavity																							
Lipoma					X																		
Skeletal Muscle																							
Focal degeneration/myositis								1															

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 48

SUMMARY OF LESIONS IN RATS FED 1% TMG FOR 24 MONTHS

Sex:	Male								Female																
Rat No.:	162	167	168	169	172	173	175	182	261	262	263	264	265	266	267	268	270	272	274	275	276	279	280	281	283	285
Treatment-Related Lesions																										
Pituitary		0																					0			
Chromophobe adenoma			X		X						X	X				X			X	X						X
Liver																										
Cholangiofibrosis	1	4	4	4	4	4	1	1	4	4	4	4	4	4	2	4	4	4	4	3	4		4	4	4	1
Cystic bile duct hyperplasia		1					1		4	1		3	1		1	3	1	1	1	3		3	2	4	2	
Adenomatoid bile duct hyperplasia		1				2			1				1			3					1		3			
Areas or foci of hepatocellular alteration					3		1	2	1	1	2		2		3		3	3	3	3	2	1	1	3	3	3
Neoplastic nodules								X							X											X
Hepatocellular carcinoma	X	X	X	X		X	X		X	X	X	X					X		X	X		X	X		X	
Spleen																										
Excessive pigmentation		1		1			1			2			3	1	1	1	1	3	3	2	1		3	1	3	2
Testis																										
Interstitial cell tumor	X	X		X	X	X	X																			
Kidney																										
Excessive pigmentation		2	2	2		1			1	1	1			3	1	1	1	1	1		1	1	2	2	3	1
Mammary Gland																										
Fibroadenoma									X																	
Other Lesions																										
Adrenal Gland																										
Cystic degeneration				1			1		1			2		1			1	1		3	2	3	2			
Cortical hyperplasia	1																									
Cortical tumor										X																
Pheochromocytoma		X								X																
Extracapsular cortical nodule	X										X															
Thyroid																		0								
C cell adenoma																	X									
Squamous metaplastic follicle	1			2		1	2	2		1	1		1													
Hyperplasia of parathyroid	1																									
Trachea																										
Tracheitis		1				4								3											1	
Lungs																										
Chronic murine pneumonia	1	3	1	1	1	1	1		1	1	1	1	1	1		1	2	1	1	1	2	1		1	1	1
Neoplastic emboli		X	X																							
Hemangiosarcoma			X																							
Bronchogenic carcinoma																										X
Heart																										
Myocardial degeneration/fibrosis		1	1				1															1				
Dilated ventricle	2											1														
Liver																										
Simple bile duct hyperplasia					1																					
Portal inflammation					1																					
Focal necrosis			1																							
Cystic degeneration	3																									
Hemangiosarcoma			X																							
Telangiectasis		1														1										

TABLE 48 (concluded)

Sex:	Male								Female												
Rat No.:	162	167	168	169	172	173	175	182	261	262	263	264	265	266	267	268	270	272	274	275	276
Other Lesions (concluded)																					
Spleen																					
Extramedullary hematopoiesis	1	1	3																		
R. E. cells hyperplasia					1	1	1														
Dilated sinusoid												3			1						
Testis																					
Atrophy of seminiferous tubules		2	4	4																	
Periarteritis nodosa		2																			
Seminoma					X																
Epididymis																					
Atrophy		2		1		1															
Epithelial vacuolization							3	1													
Prostate																					
Prostatitis			1			1															
Seminal Vesicle																					
Atrophy		3	1	2		1															
Ovary																					
Ovarian cysts									X		X			X			X	X		X	
Uterus																					
Endometritis									2		1		2				1			1	
Dilated lumen													1				1			1	
Abscessation																					
Pancreas																					
Focal acinar atrophy		1				1						1									
Islet cell hyperplasia																					
Periarteritis nodosa		1																			
Vacuolization of islet cell														3						1	
Lymph Node																					
Lymphoid depletion		1				1															
Stomach																					
Dilated crypts						1	1												1		
Intestine																					
Parasitism (pinworm infestation)		1											1				1				
Enteritis											1										
Kidney																					
Chronic senile nephropathy	1	3	2	2			1														
Hydronephrosis					1																
Microcalculi									1		1										
Tubular nephrosis						3									1	2					
Cyst														X							
Foci of mononuclear cells																					
Urinary Bladder																					
Papilloma - carcinoma											X										
Foci of mononuclear cells																				1	
Focal epithelial hyperplasia				1																1	
Skin																					
Subcutaneous mesenchymal tumor	X																				
Rib																					
Hypocellularity of bone marrow	2					1													1		

Sex:	Female																				
Rat No.:	279	280	281	283	285																
Other Lesions (concluded)																					
Spleen																					
Extramedullary hematopoiesis																					
R. E. cells hyperplasia																					
Dilated sinusoid																					
Testis																					
Atrophy of seminiferous tubules																					
Periarteritis nodosa																					
Seminoma																					
Epididymis																					
Atrophy																					
Epithelial vacuolization																					
Prostate																					
Prostatitis																					
Seminal Vesicle																					
Atrophy																					
Ovary																					
Ovarian cysts		X																			
Uterus		0																			
Endometritis	1				1																
Dilated lumen					2																
Abscessation					X																
Pancreas																					
Focal acinar atrophy																					
Islet cell hyperplasia					1																
Periarteritis nodosa																					
Vacuolization of islet cell	1			1																	
Lymph Node																					
Lymphoid depletion																					
Stomach																					
Dilated crypts	1																				
Intestine																					
Parasitism (pinworm infestation)				1																	
Enteritis																					
Kidney																					
Chronic senile nephropathy																					
Hydronephrosis																					
Microcalculi																					
Tubular nephrosis	1																				
Cyst																					
Foci of mononuclear cells		1		1																	
Urinary Bladder		0																			
Papilloma - carcinoma																					
Foci of mononuclear cells																					
Focal epithelial hyperplasia				1																	
Skin																					
Subcutaneous mesenchymal tumor	X																				
Rib																					
Hypocellularity of bone marrow																					

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present

TABLE 49

SUMMARY OF LESIONS IN RATS FED TNG FOR 24 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0						0.01				0.1							1						
Sex:	Male			Female			Female				Male				Female			Male			Female			
Rat No.:	024	025	026	070	072	073	222	224	226	230	154	155	157	158	255	258	260	184	187	190	286	288	289	
Treatment-Related Lesions[a/]																								
Pituitary																								
Chromophobe Adenoma		X		X	X		X	X		X	X					X	X					X		
Liver																								
Cholangiofibrosis																		4		4	4	4	3	
Cystic bile duct hyperplasia																				4	1		4	
Adenomatoid bile duct hyperplasia																						2		
Areas of foci of hepatocellular alteration		1			1				1		2	2		1	3	2	2	2	3		3	3	2	
Neoplastic nodules										X		X												
Hepatocellular carcinoma															X				X	X		X		
Testis																								
Interstitial cell tumor			X															X	X					
Kidney																								
Excessive pigmentation																				1		1	1	
Mammary Gland																								
Fibroadenoma				X	X		X		X	X					X	X	X							
Adenoma				X	X										X		X							
Fibroma															X		X							
Other Lesions																								
Adrenal Gland																								
Cystic degeneration	1			3	3	3	3	2	1	3					2	1	1					1		
Pheochromocytoma	X		X				X					X	X		X									
Extracapsular cortical nodules																3								
Thyroid																								
C cell hyperplasia					1	1				1	1				1									
C cell adenoma												X				X		X						
Squamous metaplastic follicles										1													1	
Hyperplasia of parathyroid	1											1	1											
Trachea																								
Tracheitis	4	2			1			1	1							3								
Lung																								
Chronic murine pneumonia		3	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	2	1	1	1	1	
Neoplastic emboli								X												X				
Pumice lung	1																							

TABLE 49 (Continued)

Dose (% in feed):	0						0.01				0.1							1					
Sex:	Male			Female			Female				Male				Female			Male			Female		
Rat No.:	024	025	026	070	072	073	222	224	226	230	156	155	157	158	255	258	260	184	187	190	286	288	289
Other Lesions (Continued)																							
Heart																							
Myocardial degeneration/fibrosis	2	1	1	1	1	1	1				1	1	1	1	1				1	1			
Dilated ventricle			3																				
Endocardiosis			3																				
Blood Vessels																							
Calcification (uremic)	3																						
Liver																							
Simple bile duct hyperplasia	1	1	1	1	1	1	1	2	1	1	2	2	1	1	1	3			1				
Portal inflammation											1					1							
Focal necrosis	1																	1					
Cystic degeneration																		2					
Telangiectasis								1						1									
Hepatocellular degeneration (vacuolization)	1				1		1			1		1	3										
Lymphoreticular tumor								X															
Spleen																							
Extramedullary hematopoiesis	4											1				1							
R.E. cell hyperplasia													1					1				1	1
Lymphoid depletion							1													1			
Lymphoreticular tumor								X															
Testis																							
Atrophy of seminiferous tubules	4																		4	4			
Periarteritis nodosa	1												2	X									
Degeneration of seminiferous tubules											2	1											
Epididymis																							
Atrophy	1																			3			
Foci of mononuclear cells														1									
Prostate			0																	0			
Atrophy	1																						
Seminal Vesicle																							
Atrophy	2																						
Seminal vesiculitis																				4			
Ovaries																							
Ovarian cysts				X			X	X														X	
Oophoritis with salpingitis																2							
Uterus				0																			
Polyp										X						X						X	
Endometritis					2					3						4	1						
Dilated lumen					1																		
Serosal cyst																	X						

TABLE 49 (Concluded)

Dose (% in feed):	0						0.01				0.1							1					
Sex:	Male			Female			Female				Male				Female			Male			Female		
Rat No.:	024	025	026	070	072	073	222	224	226	230	154	155	157	158	255	258	260	184	187	190	286	288	289
Other Lesions (Concluded)																							
Pancreas																							
Acinar cell tumor					X								X										
Focal acinar atrophy											1								1				
Islet cell tumor										X													
Islet cell hyperplasia					1	1		1									1						1
Periarteritis nodosa													3										
Vacuolization of islet cells					1	1				1											1	1	
Lymph Node																							
Lymphoid depletion			1																	3			
Stomach																							
Dilated cyst						1		1		1					1								
Calcification	3																						
Ulceration																				1			
Intestine																							
Parasitism (pinworm infestation)				1					1									1					1
Kidney																							
Chronic senile nephropathy	4	2	3								1	1	4	3				1	1		1	2	
Hydronephrosis													1										
Microcalculi				1	1	1	1	1	1	1					1	1			1				
Infarct																			1				
Foci of mononuclear cells				1		1			1							1							
Urinary Bladder										0													
Cystitis	1																			3			
Skin																							
Subcutaneous mesenchymal tumor			X																				
Rib																							
Hypocellularity of bone marrow		1					1																
Proliferation of endosteum						1																	
Hyperactivity of bone marrow						1		3															
Eye																							
Keratitis	1																						
Skeletal Muscle																							
Focal myositis														3									

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; 0 = tissue missing or could not be read; X = present.

TABLE 50

SUMMARY OF LESIONS IN MALE CONTROL RATS DYING AT UNSCHEDULED TIMES

Rat No.:	403	017	004	007	029	017	028	002	001	010	016	003	030	005	019	008	013
Week of Death:	46	60	74	74	75	78	84	89	95	95	95	95	97	97	99	103	104
Treatment-Related Lesions[a/]																	
Pituitary											0						
Chromophobe adenoma		X		X	X	X	X	X		X		X		X		X	X
Liver																	
Areas or foci of hepatocellular alteration							1	1			1						1
Neoplastic nodules									X								
Spleen																	
Excessive pigmentation				1		+			+								
Kidney																	
Excessive pigmentation															1		
Other Lesions																	
Adrenal gland								0									
Cystic degeneration											1				1	1	
Pheochromocytoma									X	X							X
Thyroid gland																	
C cell hyperplasia						1										1	1
C cell adenoma										X							
Follicular epithelium adenoma													X				
Squamous metaplastic follicle										1							
Hyperplasia of parathyroid															2		
Trachea																	
Tracheitis	1				3	1			3		2	4	1				1
Calcification of mucosa																1	
Lung																	
Chronic murine pneumonia			1	1	1	1	2	1			1			1	1	1	4
Bronchopneumonia										3		3					
Pumice lung															2	3	
Heart																	
Myocardial degeneration/fibrosis						1		1		1	1	1	1	1	2	1	
Focal epicarditis		1															
Blood Vessels																	
Calcification (uremic)															4	3	
Liver																	
Simple bile duct hyperplasia			1			1		1	1	1		1	1				1
Portal inflammation	3		1	1	1		1	1	1		1	1					1
Cystic degeneration														1			
Telangiectasis																	1
Hepatocellular degeneration (vacuolization)						2								1		1	
Spleen																	
Extramedullary hematopoiesis	1		3														
R.E. cell hyperplasia															2		1
Lymphoid depletion		1															
Splenitis (eosinophilic)	4																
Testis																	
Degeneration of seminiferous tubules										3						1	
Periarteritis nodosa					2					3				2	1	3	2
Epididymis																	
Atrophy																1	
Prostate																	
Prostatitis		3								1							
Seminal Vesicle																	
Atrophy																1	
Seminal vesiculitis		1															
Pancreas										0		0					
Periarteritis nodosa					1												
Lymph Node										0		0					
Lymphoid depletion		1															
Lymphadenitis (eosinophilic)	4																
Stomach																	
Calcification															3	3	
Intestine										0							
Parasitism (pin worm infestation)															1		
Peritonitis (eosinophilic)	4																
Mesentery																	
Periarteritis nodosa					3												
Kidney																	
Chronic senile nephropathy				1	4		1	3	3	4	1	2	1	4	4	4	3
Hydronephrosis		1						1							1		
Tubular nephrosis					1												
Pyelonephritis		3															
Skin																	
Subcutaneous mesenchymal tumor							X	X									
Epithelial tumor			X	X													
Brain																	
Astrocytoma													X				
Rib																	
Hypocellularity of bone marrow						2											
Proliferation of endosteum															1	1	
Eye																	
Keratitis					4											1	

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; + = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 51

SUMMARY OF LESIONS IN FEMALE CONTROL RATS DYING AT UNSCHEDULED TIMES

Rat No.:	451	059	071	076	057	075	062	077	066	074	055	079	080	078	052	051	069
Week of Death:	38	70	78	81	81	85	85	85	88	96	96	97	97	98	99	99	103
Treatment-Related Lesions[a/]																	
Pituitary	0																
Chromophobe adenoma		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Liver																	
Areas or foci of hepatocellular alteration							1					1	1				1
Spleen																	
Excessive pigmentation								1									
Mammary Gland																	
Fibroadenoma			X			X								X	X		X
Adenoma				X		X											
Adenomacarcinoma-carcinoma	X																
Other Lesions																	
Adrenal Gland	0																
Cystic degeneration		2	2	3	3	1	3	3	3	2	2	3		2	1	3	2
Cortical tumor													X				
Calcification																1	
Thyroid Gland	0															0	
C cell hyperplasia								1									1
C cell adenoma											X						
Hyperplasia of parathyroid			1														
Trachea																	
Tracheitis		2					1			1				1			
Lung																	
Chronic murine pneumonia		1		1	1			1	1	1		1	1	1	1	1	1
Metastatic tumor	X																
Pumice lung			1														
Heart																	
Myocardial degeneration/fibrosis			1	1				1		1					1		
Blood Vessels																	
Calcification (uremic)			1														
Liver																	
Simple bile duct hyperplasia			3	1	1	1				1	1	1	1	1			1
Portal inflammation		1			1			1									1
Focal necrosis		1	1														
Hepatocellular degeneration (vacuolization)	1	1		2		1		1	2	2			1			1	2
Spleen																	
Extramedullary hematopoiesis	4								3				1				
Ovary																	
Ovarian cyst			X		X	X	X		X					X			
Abscessation																	4
Uterus																	
Endometritis		2		1	1			1			1		4				3
Pancreas										0							
Focal acinar atrophy			4														
Islet cell hyperplasia		1			1				1			1					1
Stomach																	
Dilated crypts									1								1
Calcification			1														
Ulceration	1																
Kidney																	
Chronic senile nephropathy					1												
Microcalculi		1				1		1	1			1	1	1	1		1
Tubular nephrosis			2														
Foci of mononuclear cells						1	1					1		1			
Carcinoma			X														
Skin																	
Subcutaneous mesenchymal tumor						X											
Epithelial tumor									X								
Brain																	
Astrocytoma																	X
Rib																	
Hypercellularity of bone marrow			1														
Proliferation of endosteum			1														
Eye																	
Keratitis		1													1		
Abdominal Cavity																	
Lipoma							X										

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 52

SUMMARY OF LESIONS IN MALE RATS FED 0.01% TNG AND DYING AT UNSCHEDULED TIMES

Rat No.:	309	314	416	415	109	119	112	121	108	414	116	118	103	125	421	129	110	424	122	111	117	113	127	420
Week of Death:	27	41	44	44	59	73	80	80	82	87	88	88	88	88	92	93	96	99	101	103	103	105[b]	105[b]	108[c]
Treatment-Related Lesions[a]																								
Pituitary	0		0		0																			
Chromophobe adenoma						X			X			X	X		X	X	X	X	X			X		X
Liver																								
Areas or foci of hepatocellular alteration							1	1			1	1		1	1		2	1	1	1	3	1		
Spleen																								
Excessive pigmentation																2								
Other Lesions																								
Adrenal Gland	0		0			0																		
Cystic degeneration									1						1			1					1	
Cortical hyperplasia											1						1							
Pheochromocytoma											X		X			X								
Thyroid Gland	0																	0						
C cell adenoma													X						•					X
Follicular epithelial adenoma																							X	
Squamous metaplastic follicle			1																			1		
Hyperplasia of parathyroid								1					1	1										1
Trachea	0																							
Tracheitis		1		3	2		1							1			1			1		1		
Lung																								
Chronic murine pneumonia				3	1		1	1	1	1		1	3	2	1		1	1	1	3	1	1	3	1
Malignant lymphoma or granulocytic leukemia		X									X													
Pumice lung													3											1
Heart																								
Myocardial degeneration/fibrosis							1	2	1	1			1	1	2	1	1	1	2	1	1	1	2	1
Dilated ventricle									1							1								
Blood Vessels																								
Calcification (uremic)								3					3	3										1
Liver																								
Simple bile duct hyperplasia			1	1	1	1	1	1		2	1	1	2	1	1	2	1	2		1	1	1	2	1
Portal inflammation			1	1	1		1						1			1		1					1	1
Telangiectasis																			1					
Hepatocellular degeneration (vacuolization)						1										3						1	1	1
Malignant lymphoma or granulocytic leukemia	X	X								X	X													
Spleen																								
Extramedullary hematopoiesis				2			3	1		4			1	3			1	2						3
R. E. cell hyperplasia											1		3							2				
Malignant lymphoma or granulocytic leukemia	X	X																						
Lymphoid depletion												2												
Testis																								
Atrophy of seminiferous tubules																			4					
Degeneration of seminiferous tubules								2							4									
Periarteritis nodosa														1					3				1	

TABLE 52 (Concluded)

Rat No.:	309	314	416	415	109	119	112	121	108	414	116	118	103	125	421	129	110	424	122	111	117	113	127	420
Week of Death:	27	41	44	44	59	73	80	80	82	87	88	88	88	88	92	93	96	99	101	103	103	105[b]	105[b]	108[c]
Other Lesions																								
Epididymis																								
Atrophy															2									
Epithelial vacuolization																		2						
Epididymitis																2								
Prostate	0														0	0								
Prostatitis														4									3	
Seminal Vesicle	0																							
Atrophy								1							3	2								
Seminal vesiculitis														4	1	2		4					1	
Pancreas																								
Focal acinar atrophy					1		1			1											1		1	
Acinar cell hyperplasia											1													
Islet cell hyperplasia																	2					1		
Periarteritis nodosa																			3				3	
Vacuolization of islet cells															1									
Foci of mononuclear cells											1													
Lymph Node																								
Malignant lymphoma or granulocytic leukemia	X	X																						
Stomach																								
Dilated crypts											1			3							1			
Calcification								3					4	3		2							1	3
Papilloma																		X						
Intestine																								
Parasitism (pinworm infestation)														1			1						1	
Kidney																								
Adenocarcinoma																			X					
Chronic senile nephropathy	1		2	1		1		4			3	2	4	4	4		1		4	1	3	2	4	2
Hydronephrosis											1													
Microcalculi							2																	
Tubular nephrosis					1																			
Granulocytic leukemia or malignant lymphoma		X								X														
Cyst						1																		
Foci of mononuclear cells							1		1									1						
Urinary Bladder	0																							
Focal epithelial hyperplasia															1	3								
Cystitis															3	3		1						1
Skin																								
Subcutaneous mesenchymal tumor											X						X			X				X
Epithelial tumor							X																	
Rib	0																							
Hypocellularity of bone marrow															1	2								
Hypercellularity of bone marrow							1																	
Proliferation of endosteum								2						1										
Leukemia		X																						
Eye	0																							
Keratitis															1	1								
Uveitis																					1			
Mesentery																								
Malignant lymphoma										X														

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; 0 = tissue missing or could not be read; X = present.

b/ Died in first week of recovery after being fed TNG for 24 months.

c/ Died in fourth week of recovery after being fed TNG for 24 months.

TABLE 53

SUMMARY OF LESIONS IN FEMALE RATS FED 0.01% TRU AND DYIP / AT UNSCHEDULED TIMES

Rat No.:	212	221	228	219	204	217	227	215	208	223	213	424	225	229	216	210	211	220	467	218	472
Week of Death:	40	50	59	70	80	80	90	80	85	85	86	88	89	96	96	96	96	99	99	99	104
Treatment-Related Lesions[a/]																					
Pituitary																					
Chromophobe adenoma			X		X			X	X	X	X			X		X	X	X	X	X	X
Liver																					
Areas or foci of hepatocellular alteration											1	1			1				1		1
Spleen																					
Excessive pigmentation											1							1			
Kidney																					
Excessive pigmentation							1			1							1				
Mammary Gland																					
Fibroadenoma	X						X			X			X		X		X	X	X		
Adenoma								X				X		X							
Adenocarcinoma-carcinoma					X																
Other Lesions																					
Adrenal Gland																					
Cystic degeneration	2		1	3	1	1	2	3	3	2	3	3		2	2	1	3	4	2	1	
Pheochromocytoma																					X
Thyroid Gland			0		0		0														
C cell hyperplasia						1				1											
Trachea																					
Tracheitis			1	3				1			1		1			1	1	1	1		
Lung																					
Chronic murine pneumonia	1	1	1	3		1	1	1		1	1	1	1	1	1	1	1	1		1	1
Heart																					
Myocardia degeneration/fibrosis					1										1		1	1		1	1
Liver																					
Simple bile duct hyperplasia	1			1		1			1		1		1	1	1	1	1	1		1	
Portal inflammation										1					1						
Focal necrosis								1					1								
Telangiectasis													1								
Hepatocellular degeneration (vacuolization)					2					2						1	1	1		3	
Extramedullary hematopoiesis					1																
Spleen																					
Extramedullary hematopoiesis	1			2	4	3	2	1				2	2		2				3		
Lymphoid depletion									3												1
Ovary		0																			
Ovarian cyst	X		X			X	X							X				X			X
Uterus																					
Polyp						X															X
Endometritis						1			1	1	1					1		1		1	1
Dilated lumen					1	1	1				1							1			
Pancreas																					
Focal acinar atrophy						1															
Islet cell hyperplasia			1					1		1		1		1					1	1	
Lymph Node																					
Lymphoid hyperplasia	1																				
Thymus																					
Thymoma					X																
Stomach																					
Dilated crypts			1						1												
Intestine																					
Parasitism (pinworm infestation)			1	1	1		1								1						
Kidney																					
Hypoplasia										X											
Chronic senile nephropathy											1										
Hydronephrosis										1											
Microcalculi				1	1			1				1		1	1	1		1		1	1
Tubular naphrosis						3															
Foci of mononuclear cells						1	1									1					
Urinary Bladder																					
Foci of mononuclear cells																	1				
Cystitis														1							
Skin																					
Subcutaneous mesenchymal tumors				X								X									
Rib																					
Hypocellularity of bone marrow																	1				
Eye																					
Keratitis																				1	

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; 0 = tissue missing or could not be read; X = present.

TABLE 54

SUMMARY OF LESIONS IN MALE RATS FED 0.1% TNG AND DYING AT UNSCHEDULED TIMES

Rat No.:	133	132	139	140	152	156	149	150	151	145	139	147	153
Week of Death:	49	70	75	78	80	80	93	93	95	97	99	99	101
Treatment-Related Lesions[a/]													
Pituitary						0							
Chromophobe adenoma		X	X	X	X			X	X		X		X
Liver													
Areas or foci of hepatocellular alteration	1	1	1				1	1		1		1	2
Hepatocellular carcinoma										X			X
Spleen													
Excessive pigmentation		2	1					1	1			1	1
Testis													
Interstitial cell tumor						X							
Mammary Gland													
Fibroadenoma											X		
Other Lesions													
Adrenal Gland						0							
Pheochromocytoma							X						
Pituitary Gland						0							
Minute foci of calcification										1			
Thyroid Gland						0							
Follicular epithelium adenoma												X	
Squamous metaplastic follicle	1									1			
Trachea						0							
Tracheitis				1	1		1		3	4		3	
Lung													
Chronic murine pneumonia	1	1		1	1	1	1	1		4	2	1	1
Bronchopneumonia									1				
Heart													
Myocardial degeneration/fibrosis	1	1	1		1						1		1
Focal epicarditis					1								
Liver													
Simple bile duct hyperplasia	1		1	1	1		1		2	3	2	1	2
Portal inflammation	1	1	1				1				1		
Cystic degeneration						1					1	1	1
Telangiectasis						1		1					
Hepatocellular degeneration (vacuolization)			1	4							2		
Extramedullary hematopoiesis	1												
Spleen													
Extramedullary hematopoiesis	3												
Lymphoid depletion						4				1			
Testis													
Atrophy of seminiferous tubules			4								3		
Epididymis			0										
Atrophy						1					1		
Epithelial vacuolization											1	1	
Prostate						0		0					
Prostatitis											2		
Atrophy			1										
Seminal vesicle						0		C					
Atrophy			1										
Seminal vesiculitis											1		
Pancreas						0			0				
Focal acinar atrophy							1			1			
Islet cell hyperplasia							1	1				1	1
Foci of mononuclear cells	1												
Lymph Node						0							
Stomach						0							
Calcification										3			
Ulceration				1								1	
Intestine						0							
Enteritis											1		
Kidney													
Chronic senile nephropathy		2				4		1	1	3	1		1
Foci of mononuclear cells	1		1		1							1	
Urinary Bladder					0	0							
Skin						0							
Subcutaneous mesenchymal tumor	X												
Epidermal inclusion cyst		X											
Epithelial cell tumor												X	
Brain													
Meningitis (suppurative)												1	
Rib						0		0					
Hypocellularity of bone marrow		3		1					1		1		2
Mediastinum													
Mesenchymal cell tumor					X								

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 55

SUMMARY OF LESIONS IN FEMALE RATS FED 0.1% TNG AND DYING AT UNSCHEDULED TIMES

Rat No.:	253	269	257	265	244	237	24.	250	247	256	254	246	251	239	259	252	248	242 [1]
Week of Death:	46	70	71	71	72	80	84	85	85	86	88	89	89	92	98	98	99	100
Treatment-Related Lesions[a/]																		
Pituitary	0																	
Chromophobe adenoma		X	X	X	X		X	X		X	X	X	X	X	X	X	X	
Liver																		
Area or foci of hepatocellular alteration		1				1	2	2	2		1	1	1	1	1	1		
Spleen																		
Excessive pigmentation			1		1				1	2		1						
Kidney																		
Excessive pigmentation							1	1	1						1			
Mammary Gland																		
Fibroadenoma						X		X	X									
Adenocarcinoma-carcinoma	X																	
Other Lesions																		
Adrenal Gland																		
Cystic degeneration				3			1	1	1		2	2	1	1	2	3	1	1
Pheochromocytoma								X										
Thyroid Gland				0									0			0	0	
C cell hyperplasia		1					1											
C cell adenoma												X						X
Trachea																		
Tracheitis		1												1				
Lung																		
Chronic murine pneumonia	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Heart																		
Myocardial degeneration/fibrosis				1										1	1			
Liver																		
Simple bile duct hyperplasia	1		1					1		1							1	2
Portal inflammation		1			1	1					1	1	1	1				
Cystic degeneration							2											1
Hepatocellular degeneration (vacuolization)			1		1					1	2		1	1			1	
Pigmentation							1			1								
Ovary																		
Ovarian cyst			X		X						X	X						
Uterus																		
Endometritis									1						1			
Dilated lumen																3		
Pancreas																		
Focal acinar atrophy	1																	
Acinar cell hyperplasia							1											
Islet cell hyperplasia												1	1		1	1		
Foci of mononuclear cells													1					
Lymph Node																		
Malignant lymphoma													X					
Stomach																		
Dilated crypts														1		1		
Ulceration																	1	
Intestine																		
Parasitism (pinworm infestation)	X																	
Kidney																		
Microcalculi		1	1				1	1				1	1		1	1		1
Tubular nephrosis															3		1	
Foci of mononuclear cells	1	1	1											1				
Cyst											X							
Urinary Bladder																		
Foci of mononuclear cells										1								
Brain							0											
Rib																		
Hypocellularity of bone marrow																	1	

[1] Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable;) = tissue missing or unreadable; X = present.

b/ Died in second week of recovery after being fed TNG for 24 months.

TABLE 56

SUMMARY OF LESIONS IN RATS FED 12 TMC AND DYING AT UNSCHEDULED TIMES

Sex:	Male									Female			
Rat No.:	431	180	185	186	164	177	180	181	432	174	290	282	284	271
Week of Death:	61	75	82	63	88	92	97	97	103	103	50	72	85	89
Treatment-Related Lesions[a]														
Pituitary		0								0				
Chromophobe adenoma				X	X				X					
Liver														
Cholangiofibrosis		1	4	4	1	4	4		4	4	1	1	4	1
Cystic bile duct hyperplasia					2	1		2				1	2	
Adenomatoid bile duct hyperplasia			2	2			1							
Areas of foci of hepatocellular alteration		3	2	3	3			4	3		2		3	
Neoplastic nodules							X				X		X	X
Hepatocellular carcinoma			X			X	X	X		X				
Spleen														
Excessive pigmentation		2			3				1		2			1
Testis														
Interstitial cell tumor			X					X		X				
Kidney														
Excessive pigmentation			1	1	1		1	2		1	1	2	1	1
Mammary Gland														
Fibroadenoma											X			
Cyst														X
Other Lesions														
Thyroid Gland														
C cell adenoma									X					
Squamous metaplastic follicle					1									
Lung														
Chronic murine pneumonia		1	1	1	1		1	1	1	1	1		1	1
Neoplastic emboli	X		X				X							
Malignant lymphoma												X		
Heart														
Myocardial degeneration/fibrosis		2												
Dilated ventricle	3			1		1				1	1	2		
Liver														
Simple bile duct hyperplasia		2					3							
Portal inflammation		1			1									
Liver necrosis							4			1				
Hemangiosarcoma							X	X						
Leukemia (granulocytic)/malignant lymphoma	X											X		
Spleen														
Extramedullary hematopoiesis			1				4							
R.E. hyperplasia		1	2					?						1
Lymphoid depletion						1								
Leukemia (granulocytic)	X													
Testis														
Atrophy of seminiferous tubules			4				1		4	2				
Degeneration of seminiferous tubules						2								
Epididymis						0								
Atrophy										2				
Sperm granuloma									X					
Prostate			0	0		0		0	0					
Prostatitis										3				
Seminal Vesicle			0			0		0						
Atrophy									2	2				
Ovary														
Ovarian cyst													X	
Pancreas						0								
Focal acinar atrophy					1									
Lymph Node														
Malignant lymphoma												X		
Leukemia (granulocytic)	X													
Metastatic tumor (renal node)							X							
Stomach														
Dilated crypts			1		1								1	
Intestine														
Malignant lymphoma												X		
Parasitism (pinworm infestation)							X							
Kidney														
Chronic senile nephropathy					1			1	2	1				
Tubular nephrosis						3								
Foci of mononuclear cells	1	1												1
Cyst								X						
Malignant lymphoma/leukemia (granulocytic)	X											X		
Urinary Bladder														
Foci of mononuclear cells			1											
Skin														
Subcutaneous mesenchymal tumor								X					X	
Epithelial cell tumor		X	X											
Rib														
Leukemia	X													
Mediastinum														
Leukemia (granulocytic)	X													

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; 0 = tissue missing or unreadable; X = present.

TABLE 57

NON-TNG RELATED TUMORS IN RATS FED TNG FOR 24 MONTHS OR DYING AT UNSCHEDULED TIMES

Dose (% in feed):	0		0.01		0.1		1	
Sex:	Male	Female	Male	Female	Male	Female	Male	Female
Tumors[a/]								
Adrenal Gland								
Cortical adenoma	1	1						1
Pheochromocytoma	5	1	4	4	4	2	1	1
Thyroid Gland								
C cell adenoma	1	2	2	1	1	3	2	1
Follicular epithelium adenoma	1		1		2			
Lung								
Metastatic nodule		1		1				
Hemangiosarcoma							1	
Bronchogenic carcinoma								1
Liver								
Hemangiosarcoma							3	
Testis								
Seminoma							1	
Pancreas								
Acinar cell tumor		1			1			
Islet cell tumor				1				
Lymph Node								
Hemangioma			1					
Thymus Gland								
Thymoma				1				
Multiple Sites								
Hematopoietic cell tumors			4	3	1	1	1	1
Stomach								
Papilloma			1					
Kidney								
Adenoma		1						
Adenocarcinoma			1		1			
Carcinoma		1						
Bladder								
Papilloma-carcinoma								2
Skin								
Epithelial tumor	2	2	1		1		2	
Subcutaneous mesenchymal tumor	3	1	4	2	1	1	2	2
Brain								
Astrocytoma	1	1			1			
Body Cavities								
Lipoma		2						
Mesenchymal cell tumor					1			

a/ Number of rats with the tumor.

TABLE 58

INCIDENCE OF TNG-RELATED LESIONS IN RATS FED TNG FOR 24 MONTHS OR DYING AT UNSCHEDULED TIMES

Dose (% in feed):	0		0.01		0.1		1	
Sex:	Male	Female	Male	Female	Male	Female	Male	Female
Lesions[a/]								
Pituitary								
Chromophobe adenoma	14/23(61)[a/]	22/28(79)	12/25(48)	21/32(66)	14/25(56)	21/27(78)	5/19(26)	7/24(29)
Liver								
Cholangiofibrosis	0/24	0/29	0/28	0/32	0/26	0/28	18/21(86)	24/25(96)
Cystic bile duct hyperplasia	0/24	0/29	0/28	0/32	0/26	0/28	6/21(29)	18/25(72)
Adenomatoid bile duct hyperplasia	0/24	0/29	0/28	0/32	0/26	0/28	5/21(24)	6/25(24)
Areas or foci of hepatocellular alteration	6/24(25)	7/29(24)	14/28(50)	8/32(25)	17/26(65)	20/28(71)	11/21(5])	20/25(80)
Neoplastic nodules	1/24(4)	0/29	0/28	1/32(3)	1/26(4)	1/28(4)	2/21(10)	5/25(20)
Hepatocellular carcinoma	0/24	0/29	0/28	0/32	3/26(12)	2/28(7)	13/21(62)	11/25(44)
Spleen								
Excessive pigmentation	3/24(13)	1/29(3)	2/28(7)	2/32(6)	6/26(23)	5/28(18)	6/21(29)	16/25(64)
Testis								
Interstitial cell tumor	2/24(8)	--	1/28(4)	--	3/26(12)	--	11/21(52)	--
Kidney								
Excessive epithelial pigmentation	1/24(4)	0/29	0/28	3/32(9)	0/26	3/28(11)	6/21(29)	21/25(84)
Mammary Gland								
Tumor (any type)	1/24(4)	13/29(45)	0/28	19/32(59)	1/26(4)	12/28(43)	0/21	2/25(8)
Fibroadenoma	1/1(100)[b/]	11/13(85)	--	14/19(74)	1/1(100)	8/12(67)	--	2/2(100)
Adenoma	0/1	6/13(46)	--	3/19(16)	0/1	5/12(42)	--	0/2
Fibroma	0/1	0/13	--	0/19	0/1	2/12(17)	--	0/2
Adenocarcinoma-carcinoma	0/1	1/13(8)	--	2/19(11)	0/1	2/12(17)	--	0/2

a/ Rats with lesions/rats with readable slides (percent incidence).
b/ Figures for rats with mammary tumors.

TABLE 59

AGE, WEIGHT AND FERTILITY OF THREE GENERATIONS OF RATS GIVEN TNG

TNG (% in feed)	Generation	Age at First Mating (months)	Males: Mating Ratio	Males: Pregnancy Ratio	Males: Fertile Mated	Males: Weight (g) at First Mating	Females: Fertile Mated	Females: Weight (g) at First Mating	Duration of Gestation (days)
0	F0	5	41/48[a]	37/41[b]	10/10	608 ± 15[c]	23/24	305 ± 4[c]	22.6
	F1	5	35/40	24/35	17/20	590 ± 12	15/20	308 ± 5	22.3
	F2	4	35/40	31/35	19/20	538 ± 8	19/20	293 ± 6	22.1
0.01	F0	5	39/47	33/39	9/10	649 ± 21	19/24[d]	328 ± 5[e]	23.0
	F1	5	36/40	32/36[d]	19/20	669 ± 10[e]	19/20[d]	328 ± 8	22.4
	F2	4	33/39	28/33	18/20	555 ± 10	17/20	321 ± 9[e]	22.2
0.1	F0	5	43/46	37/43	10/10	604 ± 22	24/24	309 ± 4	22.7
	F1	5	36/40	32/36[d]	20/20	543 ± 10	20/20[d]	308 ± 5	22.3
	F2	4	38/40	37/38	19/20	520 ± 8	20/20	288 ± 6	22.0
1.0	F0	5	41/47	36/41	9/9	433 ± 18[e]	23/24	247 ± 4[e]	22.9
	F1	5	35/40	8/35[d]	6/20[d]	337 ± 9[e]	8/20[d]	217 ± 5[e]	22.4
	F2[f]	4	22/28	1/22[d]	1/14[d]	336 ± 11[e]	1/14[d]	225 ± 6[e]	23.0

a/ Number of copulations detected by vaginal smear to the number of male-female pairings.
b/ Number of confirmed pregnancies to the number of copulations.
c/ Mean ± standard error.
d/ Significantly different from the ratio for the respective control generation (Fisher's exact probability test).
e/ Significantly different from the mean value of the respective control generation (Dunnett's multiple comparison procedure).
f/ Derived from first litters of the F1 generation.

TABLE 60

REPRODUCTIVE PERFORMANCE OF FEMALE RATS GIVEN TNG IN A THREE-GENERATION STUDY

TNG (% in Feed)	Litter No.	Litter Size	Live-born Index	Birth Weight	Viability Index	Lactation Index	Weight at Weaning	Males:Total at Weaning	Feed Intake (g) During Gestation
0	F1a	11.3 ± 0.7(17)[a]	96 ± 2	7.6 ± 0.2	99 ± 1	93 ± 6	51 ± 3	83:167	
	F1b	11.5 ± 1.0(13)	98 ± 2	7.8 ± 0.3	99 ± 1	85 ± 6	49 ± 3	58:121	627 ± 51
	F2a	13.9 ± 0.5(13)	100	6.7 ± 0.2	100	98 ± 1	41 ± 2	98:178	
	F2b	15.2 ± 0.5(8)	100	7.1 ± 0.2	99 ± 1	84 ± 10	41 ± 2	32:103	
	F3a	13.8 ± 0.8(15)	100	7.1 ± 0.2	99 ± 1	99 ± 1	43 ± 2	87:202	
	F3b	13.1 ± 1.4(12)	98 ± 2	7.1 ± 0.2	97 ± 4	89 ± 8	36 ± 4	77:139	
0.01	F1a	10.8 ± 0.9(16)	83 ± 8	6.9 ± 0.7	94 ± 11	83 ± 10	47 ± 6	67:131	
	F1b	13.1 ± 0.7(8)	99 ± 1	7.8 ± 0.3	97 ± 2	85 ± 8	42 ± 2	39:88	621 ± 27
	F2a	12.9 ± 0.1(16)	99 ± 1	7.3 ± 2[b]	99 ± 1	93 ± 6	43 ± 4	97:198	
	F2b	12.9 ± 1.5(13)	97 ± 3	7.0 ± 0.2	93 ± 8	88 ± 4	47 ± 3	82:142	
	F3a	13.6 ± 0.9(14)	98 ± 2	7.8 ± 0.4	100 ± 5	99 ± 1	47 ± 3	88:179	
	F3b	15.8 ± 0.9(11)	98 ± 2	6.8 ± 0.1	94 ± 3	90 ± 5	40 ± 2	74:142	
0.1	F1a	9.9 ± 0.9(20)	89 ± 5	7.9 ± 0.2	75 ± 10	94 ± 5	53 ± 2	81:158	
	F1b	11.1 ± 1.1(13)	95 ± 4	8.1 ± 0.3	97 ± 2	89 ± 4	48 ± 3	62:117	591 ± 20
	F2a	11.7 ± 1.0(19)	98 ± 1	7.1 ± 0.1	98 ± 1	98 ± 1	46 ± 3	97:206	
	F2b	14.1 ± 1.1(12)	94 ± 3	6.9 ± 0.2	97 ± 1	95 ± 2	42 ± 2	68:147	
	F3a	13.4 ± 0.7(19)	98 ± 1	7.1 ± 0.2	98 ± 1	99 ± 1	42 ± 2	110:243	
	F3b	14.8 ± 0.4(15)	98 ± 1	6.8 ± 0.1	100 ± 1	96 ± 1	39 ± 1	104:208	
1.0	F1a	6.9 ± 0.9(14)[b]	59 ± 11[c]	5.9 ± 0.2[c]	35 ± 11[c]	35 ± 12[c]	10 ± 3[c]	13:30	
	F1b	9.7 ± 0.9(9)	99 ± 1	5.8 ± 0.2[b]	75 ± 8[c]	67 ± 12	26 ± 4[b]	13:37	409 ± 20[b]
	F2a	7.0 ± 1.5(3)[c]	83 ± 17	5.4 ± 0.2[b]	83 ± 17	84 ± 8	30 ± 7	8:14	

[a] Mean ± standard error and in parentheses the number of litters included in the mean.

[b] Significantly different from the mean value of the respective control litters (Tukey's omega procedure).

[c] Significantly different from the mean value of the respective control litters (two sample rank test).

TABLE 61

REPRODUCTIVE PERFORMANCE OF FEMALE RATS GIVEN TNG IN FEED AND MATED WITH UNTREATED MALES

	% TNG in Feed			
	0	0.01	0.1	1.0
Mated[a]	21	21	20	22
Sperm positive[b]	17	11	15	20
Pregnant	15	9	12	19
Maternal weight day 0	349 ± 6[c]	343 ± 9	346 ± 8	264 ± 4[d]
Corrected weight change[e]	47 ± 6	63 ± 3	40 ± 7	25 ± 4[d]
Liver weight	15.7 ± 0.5	16.4 ± 0.8	15.7 ± 0.7	18.2 ± 0.8[d]
Relative to corrected weight[f]	4.0 ± 0.1	4.0 ± 0.2	3.8 ± 0.4	6.3 ± 0.2[d]
Implants/dam	11.6 ± 1.1	13.7 ± 1.4	11.0 ± 1.2	12.8 ± 0.7
Viable fetuses (%)[g]	89 ± 4	84 ± 5	98 ± 4	93 ± 3
Dead fetuses (%)[g]	0	0	0	0
Early resorptions (%)[g]	10 ± 4	9 ± 3	6 ± 4	3 ± 1
Late resorptions (%)[g]	1.3 ± 0.9	7.6 ± 3.8	0.6 ± 0.6	4.3 ± 1.8
Dams with complete resorptions	0	0	0	0
Live litters	15	9	12	19
Fetuses/dam	10.4 ± 1.2	11.6 ± 1.5	10.2 ± 1.3	11.8 ± 0.7
Males (%)[g]	45 ± 3	48 ± 6	53 ± 5	46 ± 4
Fetal weight (g)	2.77 ± 0.28	3.20 ± 0.18	3.25 ± 0.09	2.81 ± 0.08
Soft Tissue Anomalies				
Diaphragmatic hernia (%)[g]	0	0	0	3.7 ± 1.7
Skeletal Anomalies				
Hyoid bone				
Unossified (%)[g]	2 ± 1	4 ± 3	3 ± 3	29 ± 7[h]
Incompletely ossified (%)[g]	3 ± 3	6 ± 5	0 ± 0	19 ± 5[h]
Sternebra				
Unossified (%)	78 ± 8	65 ± 14	71 ± 11	97 ± 3

a/ Exposed to females.
b/ Sperm found in the vaginal smear.
c/ Mean or mean ± standard error.
d/ Significantly different from control (Dunnett's multiple comparison procedure).
e/ Dam body weight (day 20 - day 0) - uterine weight on day 20.
f/ Gram of liver/100 g corrected body weight (day 0 weight + corrected weight change).
g/ Mean ± S.E. of the percent of fetuses with the indicated characteristic calculated on a per litter basis.
h/ Significantly different from control ($p < 0.05$, two-sample rank test).

TABLE 62

CHROMOSOMES DERIVED FROM RATS FED TNG FOR 24 MONTHS

Dose (% in feed)	Tissue Cultured	Number of Rats	Chromosome Frequency ≤40	41	42	43	≥44	Tetraploids per 100 cells
0	Bone marrow	4	1	2	45	1	1	0.62 ± 0.31[a/]
0	Kidney	5	6	4	38	1	1	0.80 ± 0.30
1	Bone marrow	6	1	2	44	2	1	0.66 ± 0.17
1	Kidney	6	4	4	40	1	1	0.48 ± 0.21

a/ Mean ± standard error.

TABLE 63

MORPHOLOGICAL ABERRATIONS OF CHROMOSOMES
DERIVED FROM RATS FED TNG FOR 24 MONTHS

Dose (% in feed)	Tissue Cultured	Number of Rats	Chromatid Breaks and Gaps per 50 cells	Translocations per 50 cells	Total Aberrations per 50 cells
0	Bone marrow	4	0.6 ± 0.4[a/]	0.0	0.6 ± 0.4
0	Kidney	5	1.6 ± 0.5	0.0	1.6 ± 0.5
1	Bone marrow	6	1.4 ± 0.3	0.0	1.4 ± 0.3
1	Kidney	6	2.9 ± 0.7	0.1 ± 0.1	3.0 ± 0.7

a/ Mean ± standard error.

TABLE 64

REPRODUCTIVE PERFORMANCE OF MALES FED TNG FOR THE DOMINANT LETHAL MUTATION STUDY

Dose (% in feed):	0	0.01	0.1	1
Males				
Mated[a]	10	10	10	10
Fertile[b]	9	8	10	8
Females				
Receptive[c]	25	25	22	24
Mated[d]	17	15	19	17
Pregnant	17	14	17	15
Complete Resorptions	1	0	2	0
Corpora lutea/dam	15.0 ± 0.7[e]	13.8 ± 0.8	13.5 ± 0.8	16.1 ± 0.5
Total implants/dam	12.4 ± 1.0	12.1 ± 0.8	11.9 ± 1.1	14.1 ± 0.7
Viable implants/dam	11.4 ± 1.0	11.2 ± 1.0	10.8 ± 1.3	13.0 ± 1.0
Indexes				
Fertility[f]	100 (80-100)	93 (68-100)	89 (67-99)	88 (64-99)
Gestation[g]	94 (71-100)	100 (77-100)	88 (64-99)	100 (78-100)
Implantation[h]	81 ± 5	89 ± 5	85 ± 6	88 ± 4
Implant viability[i]	87 ± 6	90 ± 5	81 ± 8	92 ± 5

a/ Exposed to females.
b/ Evidence of conception found in at least one female.
c/ Proestrous females progressing into estrous overnight.
d/ Sperm found in vaginal smear.
e/ Mean ± standard error.
f/ Confirmed pregnancies/plug positive females x 100 (95% confidence limits).
g/ Pregnancies with viable embryos/confirmed pregnancies x 100 (95% confidence limits).
h/ Implants/corpora lutea x 100. Mean ± standard error.
i/ Viable embryos/implants x 100. Mean ± standard error.

TABLE 65

DISTIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS 24 HR AFTER ORAL ADMINISTRATION OF TNG-1,3-^{14}C FOLLOWING 3 MONTHS OF TNG IN FEED

	% of Administered Dose			
	Controls		1% TNG in Feed	
	Males	Females	Males	Females
G.I. Tract Plus Contents	4.5 ± 1.5[c]	4.6 ± 0.2	4.3 ± 1.3	3.7 ± 0.5
Feces	29.3 ± 4.8	7.1 ± 4.6	17.5 ± 5.0	22.8 ± 1.3
Air	21.2 ± 1.7	32.5 ± 5.2	18.6 ± 1.4	25.6 ± 3.4
Urine	14.0 ± 3.9	29.8 ± 6.7	31.7 ± 10.4	28.0 ± 10.5
Blood[a]	1.2 ± 0.2	1.0 ± 0.2	0.9 ± 0.1	1.1 ± 0.1
Spleen	<0.1	<0.1	<0.1	0.1 ± 0.0
Liver	5.5 ± 0.7	5.8 ± 1.1	5.8 ± 0.4	6.5 ± 1.1
Kidney	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
Brain	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Lungs	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
Muscle[b]	4.0 ± 0.7	4.8 ± 1.2	5.1 ± 0.8	4.7 ± 0.3
Gonads	0.1 ± 0.0	<0.1	0.2 ± 0.0	<0.1
Recovery	80.3 ± 1.6	86.4 ± 9.4	84.7 ± 4.5	94.1 ± 11.5

a/ Based on 7% of body weight.
b/ Based on 40% of body weight.
c/ Mean ± standard error of three rats.

TABLE 66

URINARY METABOLITES OF TNG 24 HR AFTER ORAL ADMINISTRATION OF TNG-1,3-^{14}C FOLLOWING 3 MONTHS OF TNG IN FEED

	% of Urinary Radioactivity			
	Controls		1% TNG in Feed	
	Males	Females	Males	Females
TNG	0.0 ± 0.0[b]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1,3-DNG	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
1,2-DNG	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
MNGS	14.4 ± 1.1	21.2 ± 1.4	12.8 ± 1.9	22.0 ± 4.1
1,3-DNG-glucuronides	8.1 + 1.0	10.5 ± 1.8	12.5 ± 1.9	10.1 ± 2.9
1,2-DNG-glucuronides	26.7 ± 3.2	24.9 ± 3.4	30.9 ± 3.6	23.1 ± 6.8
MNG-glucoronides	10.1 ± 0.7	5.8 ± 0.8	6.8 ± 0.7	4.9 ± 0.4
Glycerin	17.4 ± 2.2	14.9 ± 5.9	17.1 ± 2.7	22.0 ± 4.7
Others[a]				
Rf 0.0	19.8 ± 1.3	20.2 + 1.9	19.4 ± 0.7	17.4 ± 1.5
Rf 0.6	2.7 ± 0.8	2.1 ± 1.2	< 0.1	0.1 ± 0.1

a/ Unidentified polar components with n-butanol:acetic acid:water (50:10:40, v/v/v) solvent system.

b/ Mean ± standard error of three rats.

TABLE 67

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS 24 HR AFTER ORAL ADMINISTRATION OF TNG-1,3-^{14}C FOLLOWING 12 MONTHS OF TNG IN FEED

	% of Administered Dose			
	Controls		% TNG in Feed	
	Males	Females	Males	Females
G.I. Tract Plus Contents	6.5 ± 1.0[c/]	5.4 ± 0.9	9.1 ± 5.6	6.0 ± 3.2
Feces	17.3 ± 9.8	17.1 ± 6.1	17.5 ± 5.4	12.5 ± 5.2
Urine	36.1 ± 9.2	26.4 ± 12.9	42.3 ± 4.5	39.2 ± 6.9
Expired Air	18.1 ± 3.7	28.2 ± 1.9	14.4 ± 1.9	26.6 ± 6.7
Blood[a/]	2.8 ± 0.4	1.6 ± 0.3	1.4 ± 0.1	4.7 ± 1.7
Spleen	0.3 ± 0.2	0.1 ± 0.0	<0.1	0.1 ± 0.0
Liver	7.5 ± 7.3	6.0 ± 0.4	6.1 ± 1.5	9.4 ± 1.9
Kidneys	0.6 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
Brain	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.3
Lungs	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
Muscle[b/]	12.2 ± 3.9	5.3 ± 0.7	4.5 ± 0.6	5.8 ± 3.7
Gonads	0.2 ± 0.0	<0.1	0.2 ± 0.0	<0.1
Recovery	101.8 ± 5.4	91.0 ± 5.4	94.8 ± 6.7	105.5 ± 11.6

a/ Based on 7% of body weight.
b/ Based on 40% of body weight.
c/ Mean ± standard error of three rats.

TABLE 68

URINARY METABOLITES OF TNG 24 HR AFTER ORAL ADMINISTRATION OF TNG-1,3-^{14}C FOLLOWING 12 MONTHS OF TNG IN FEED

	% of Urinary Radioactivity			
	Controls		1% TNG in Feed	
	Males	Females	Males	Females
TNG	0.1 ± 0.1[a/]	0.7 ± 0.7	0.4 ± 0.2	0.2 ± 0.1
1,3-DNG	13.2 ± 11.7	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.1
1,2-DNG	1.2 ± 0.3	0.3 ± 0.1	0.6 ± 0.4	0.8 ± 0.5
MNGs	14.2 ± 0.8	21.6 ± 5.5	10.0 ± 1.1	14.9 ± 1.9
1,3-DNG-glucuronides	6.6 ± 0.2	6.0 ± 1.4	17.5 ± 0.4	9.5 ± 2.4
1,2-DNG-glucuronides	18.4 ± 3.2	17.7 ± 4.8	27.4 ± 4.5	24.5 ± 2.8
MNG-glucuronides	10.9 ± 2.1	6.9 ± 0.9	11.4 ± 2.9	9.2 ± 1.0
Glycerin	9.2 ± 0.6	12.1 ± 3.8	8.2 ± 3.0	9.2 ± 2.7
Unidentified	26.3 ± 8.0	34.5 ± 3.7	24.2 ± 2.8	29.5 ± 0.6

a/ Mean ± standard error of three rats.

TABLE 69

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS 24 HR AFTER ORAL ADMINISTRATION OF TNG-1,3-^{14}C FOLLOWING 24 MONTHS OF TNG IN FEED

	% of Administered Dose			
	Controls		1% TNG in Feed	
	Males	Females	Males	Females
G.I. Tract Plus Contents	1.1[c]	4.1 ± 0.1[d]	4.3 ± 0.6	3.4 ± 0.4
Feces	15.0	4.8 ± 1.4	15.9 ± 1.3	14.0 ± 4.4
Urine	26.0	36.4 ± 5.0	41.1 ± 4.0	41.2 ± 8.2
Expired Air	26.1	24.1 ± 3.2	20.7 ± 4.5	34.5 ± 4.0
Blood[a]	3.3	3.0 ± 1.0	1.6 ± 0.1	1.1 ± 0.1
Spleen	0.1	<0.1	<0.1	0.1 ± 0.1
Liver	5.8	7.0 ± 1.0	7.7 ± 0.5	7.6 ± 1.0
Kidney	2.0	0.6 ± 0.2	0.6 ± 0.1	0.4 ± 0.0
Brain	0.2	0.1 ± 0.0	<0.1	0.1 ± 0.0
Lungs	0.2	0.6 ± 0.2	0.2 ± 0.1	0.1 ± 0.0
Muscle[b]	8.8	7.4 ± 1.5	6.9 ± 0.8	6.0 ± 1.0
Gonads	0.2	<0.1	0.1 ± 0.0	<0.1
Recovery	88.8	88.7 ± 4.8	99.5 ± 5.6	108.4 ± 8.2

a/ Based on 7% of body weight.
b/ Based on 40% of body weight.
c/ Data from one rat.
d/ Mean ± standard error of three rats.

TABLE 70

URINARY METABOLITES OF TNG 24 HR AFTER ORAL ADMINISTRATION OF TNG-1,3-^{14}C FOLLOWING 24 MONTHS OF TNG IN FEED

	% of Urinary Radioactivity			
	Controls		High Dose (1% TNG)	
	Males	Females	Males	Females
TNG	<0.1[a]	0.2 ± 0.1[b]	0.6 ± 0.3	<0.1
1,3-DNG	1.4	2.6 ± 2.2	6.2 ± 4.9	0.3 ± 0.1
1,2-DNG	1.6	6.3 ± 4.6	8.0 ± 6.1	0.5 ± 0.2
MNGs	9.7	8.4 ± 0.9	14.5 ± 3.1	17.6 ± 2.2
1,3-DNG-glucuronides	8.5	8.6 ± 2.4	10.9 ± 5.3	7.4 ± 1.3
1,2-DNG-glucuronides	8.9	19.3 ± 5.8	13.5 ± 6.6	16.2 ± 1.7
MNG-glucuronides	19.1	16.9 ± 3.9	21.1 ± 2.6	10.9 ± 5.2
Glycerin	15.0	15.2 ± 5.0	14.9 ± 4.0	16.4 ± 1.6
Unidentified	36.0	22.4 ± 2.4	11.5 ± 6.1	30.5 ± 1.5

a/ One rat.
b/ Mean ± standard error of three rats.

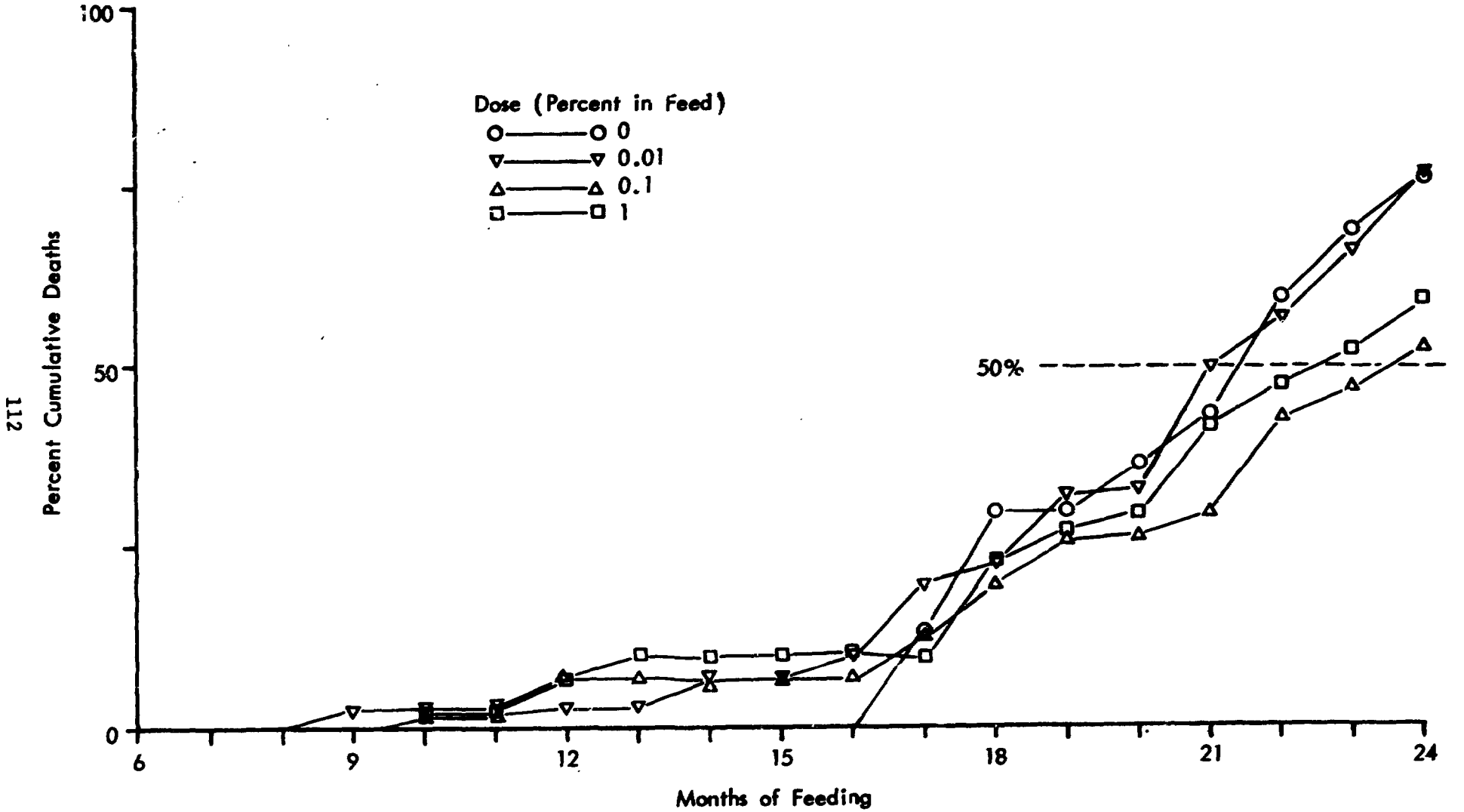


Figure 4 - Cumulative Deaths Among Male Rats Fed TNG

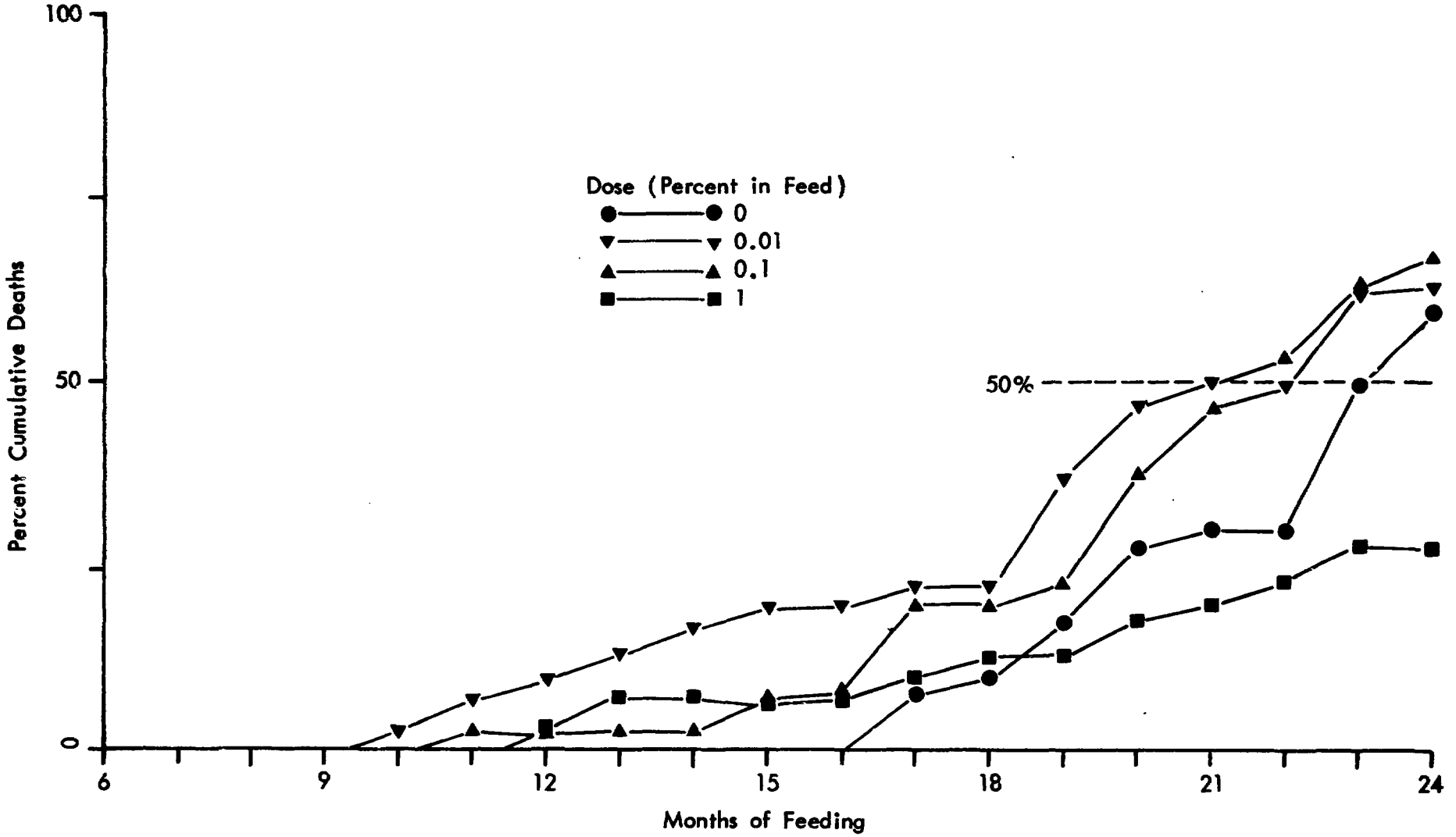


Figure 5 - Cumulative Deaths Among Female Rats Fed TNG

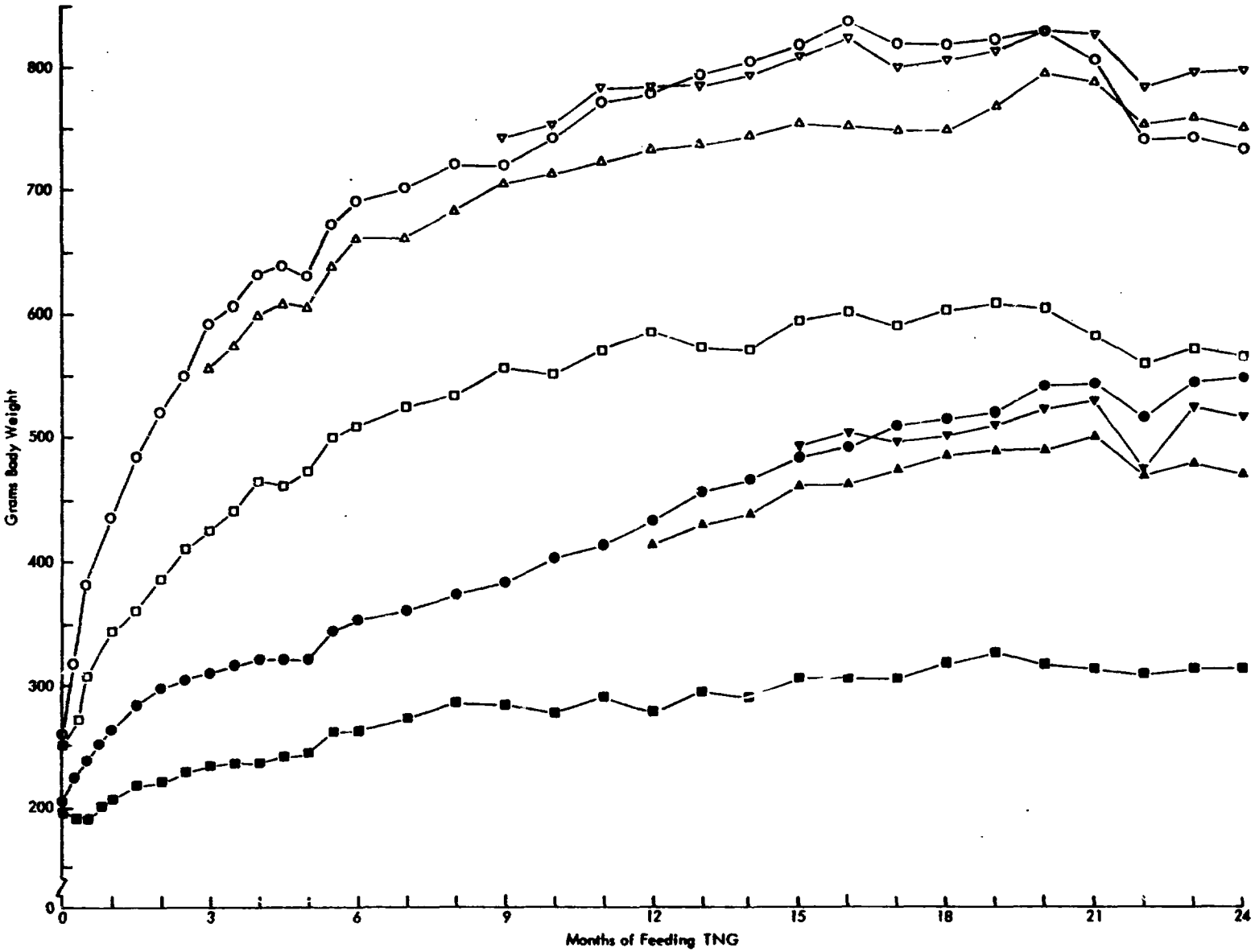


Figure 6 - Average Body Weights of Rats Fed Various Doses of TNG

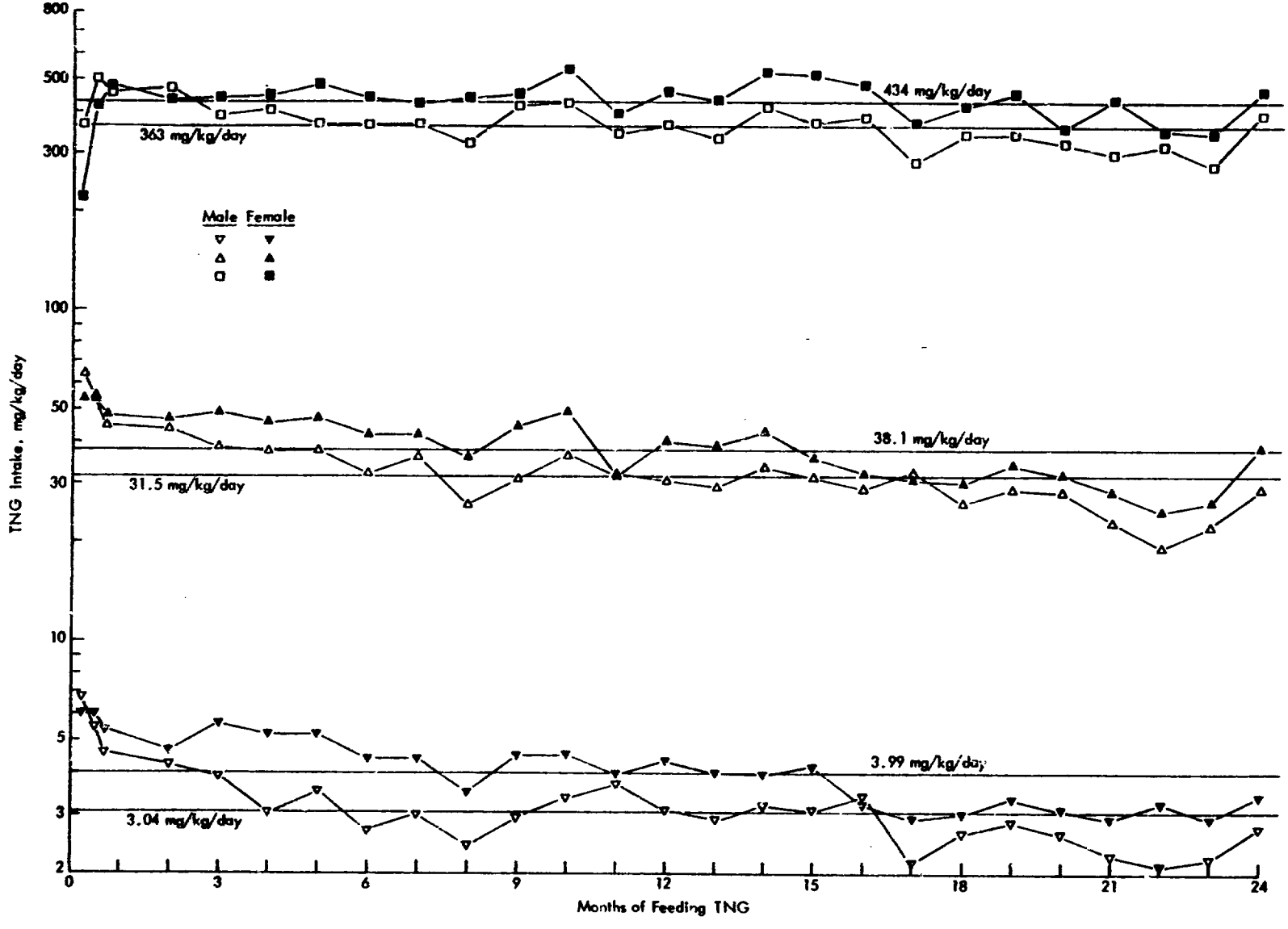


Figure 7 - Intake of TNG by Rats Fed 1% (high), 0.1% (middle) or 0.01% TNG. Horizontal Lines Are Average Intakes for Males (lower line of pair) and Females

Figure 8 - Photograph of Liver from Rat No. 83-379 Fed 1% TNG for 12 Months and Allowed to Recover for 1 Month. Note the variable-sized white patches on the liver - cholangiofibrosis.

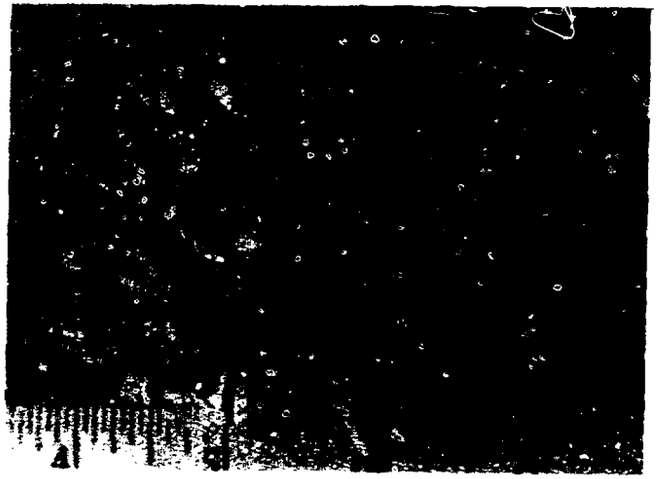


Figure 9 - Photograph of Liver from Rat No. 83-180 Fed 1% TNG for 97 Weeks. Note the large white nodular mass from caudate lobe of liver - cholangiofibrosis plus hepatocellular carcinoma.

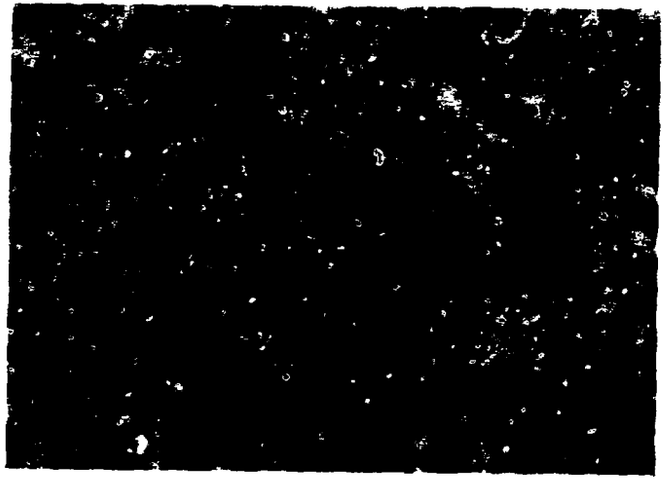


Figure 10 - Photomicrograph of Liver from Rat No. 83-379 Fed 1% TNG for 12 Months and Allowed to Recover for 1 Month. Note the earliest stage of cholangiofibrosis. H and E stain, 250 X.

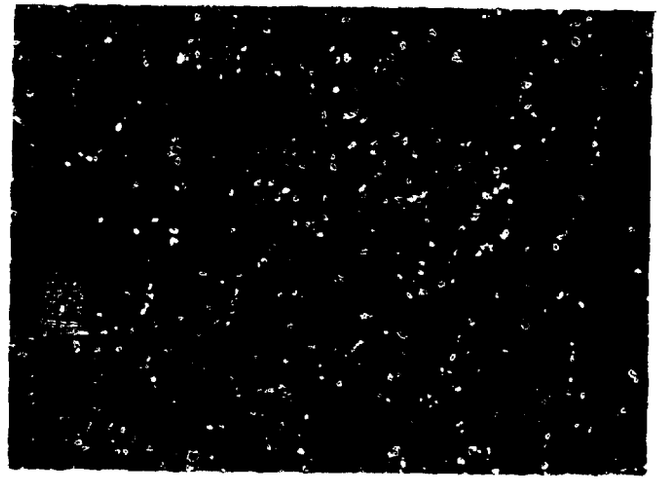


Figure 11 - Photomicrograph of Liver from Rat No. 83-379 Fed 1% TNG for 12 Months and Allowed to Recover for 1 Month. Note the cholangiofibrosis in portal area. H and E stain, 100 X.

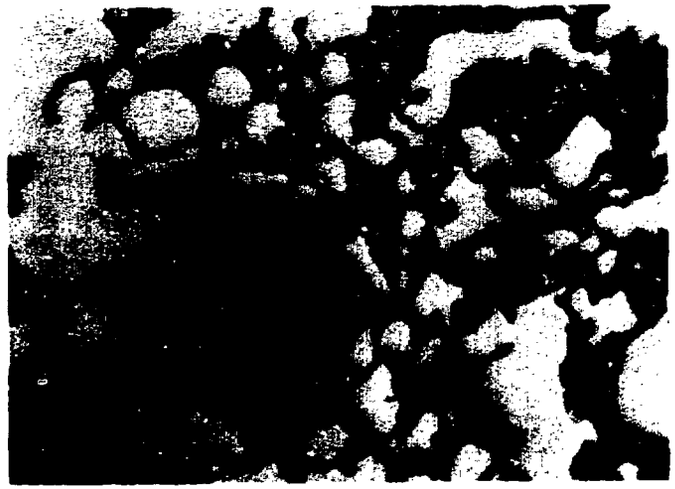
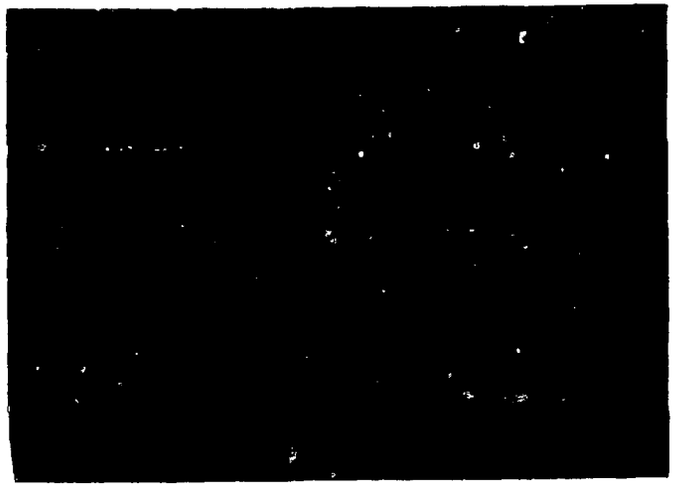


Figure 12 - Photomicrograph of Lung from Rat No. 83-180 Fed 1% TNG for 97 Weeks. Note the clusters of metastasized neoplastic cells from hepatocellular carcinoma. H and E stain, 100 X.

Figure 13 - Photomicrograph of Testis from Rat No. 83-169 Fed 1% TNG for 24 Months. Note the proliferation of interstitial cells - interstitial cell tumor. H and E stain, 250 X.



Figur 14 - Photomicrograph of Liver from Rat No. 83-169 Fed 1% TNG for 24 Months. Note the advanced stage of cholangiofibrosis. H and E stain, 100 X.

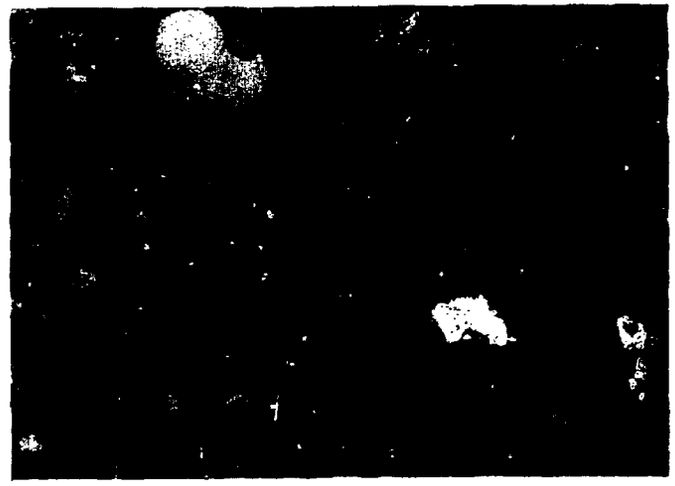


Figure 15 - Photomicrograph of Liver from Rat No. 83-190 Fed 1% TNG for 24 Months and Allowed to Recover for 1 Month. Note the glandular arrangement of liver cells - hepatocellular carcinoma. H and E st in, 250 X.

V. MOUSE STUDIES

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V. MOUSE STUDIES

The results and interpretation of various studies in mice are described below.

A. Observations and Toxic Signs

There were few untoward events among the mice. The usual fights among the males were controlled by keeping the aggressive mice in separate cages. By month 5, most of the high-dose mice had tannish, matted fur indicating a lack of normal grooming. This continued to the end of the study.

Unscheduled deaths among males and females are shown in Figures 16 and 17, respectively. Many of these mice, like the first one, control male No. 80-142, died in the night without premonitionary signs. The tissues were lost to autolysis, which occurs much more rapidly in mice than in larger animals. Cannibalization by cage mates also destroyed tissues before we could do our histopathological analysis.

Although there were usually a few more deaths in the high-dose group than in any of the others, the differences among groups were small. More significant was the pronounced sex difference in survival: females had a 2- or 3-month longer life span, as seen by the time at which half the mice died. As a result of this, very few males (and no high-dose males) were alive at the end of the 24th month.

During months 19 and 20, there was a small epidemic of a dermatosis. The etiology was uncertain, but a fungus was probable. Cases were scattered among the cages, not concentrated in a few, so individual resistance was a significant factor. The victims (or their cage mates) would scratch at the lesion, sometimes producing a large, ulcerated wound requiring euthanasia. Generally, isolation and chemotherapy produced a rapid cure. We used Tab-eze® (Haver-Lockhart Laboratories, Shawnee, Kansas), a shotgun formulation containing colloidal sulfur, mercaptobenzothiazole, tannic acid and hexylresorcinol in a soothing ointment base for treatment of local dermatitis.

B. Body Weights

Body weights of male and female mice fed TNG are shown in Figures 18 and 19, respectively. Control mice gained weight quickly, then oscillated around 46 g for males or 38 g for females after 6 months. After 16 months, there was a downward trend in the body weights of the surviving males. The

relatively large oscillations in the later months were related to individual mice losing weight shortly before death.

Body weights of male and female mice fed the low (0.01% TNG in feed) or middle (0.1% TNG) dose were similar to those of the control mice. Parts of the curves were omitted to improve clarity of the graph. Usually, the control males and low-dose females were the heaviest groups in their sexes.

Mice fed the high dose (1% TNG) lost weight during the first week or two. Thereafter, they gained weight. However, they were consistently lighter than the other groups with average weights oscillating around 40 g for males and 32 g for females, about 6 g below the control mice.

C. Feed Consumption and Compound Intake

Average feed consumption, as summarized in Table 71, was generally lower in the high-dose mice, although this was not statistically significant. There were month-to-month variations.

The monthly and overall average intakes of TNG are shown in Figure 20 and summarized in Table 71. There were some fluctuations from the means. The TNG intakes during the entire study averaged 11.10, 114.6 and 1022 mg/kg/day for the low-, middle-, and high-dose males, respectively; and averaged 9.72, 96.4 and 1058 mg/kg/day for the low-, middle- and high-dose females. The differences between the males and females at the respective dose levels are not statistically different.

D. Laboratory Data

Laboratory data from mice killed at unscheduled times are shown in Table 72. Although the values were not consistent, the high-dose (1% TNG in feed) mice usually had anemia with reticulocytosis and often had Heinz bodies. The small amounts of methemoglobin present in some mice might be artifacts inherent in the method.

Laboratory data from male and female mice fed TNG for 12 months are shown in Tables 73 and 74. The results were similar to those seen in unscheduled mice. In addition, the erythropoietic system in the high-dose mice had compensated for the toxic hemolysis implied by the presence of Heinz bodies. One control mouse, No. 80-204, had the worst anemia (5.49×10^6 erythrocytes per mm^3) and the highest reticulocyte count (7.81% of erythrocytes), BUN (59 mg %) and SGPT (80 I.U./liter) of all mice studied at that time.

Laboratory data from mice fed TNG for 24 months are shown in Tables 75 and 76. There were only a few male survivors. Some males were so vexing that they died the night before the necropsy. In the females, effects were seen in both middle (0.1% TNG) and high-dose mice. The worst anemias were middle-dose females Nos. 82-623 (2.75×10^6 erythrocytes per mm^3) and No. 82-640 (3.86×10^6); No. 82-623 also had the extreme reticulocytosis (14.89%). Both these two females had marked poikilocytosis, anisocytosis and polychromasia. Since No. 82-623 had no Heinz bodies, while No. 82-640 had only 0.36%, it is likely that this anemia was a consequence of old age rather than TNG. While the high-dose mice had milder anemia ($4.00-5.82 \times 10^6$ erythrocytes per mm^3) and mild reticulocytosis (1.28-4.28%), all had Heinz bodies (1.37-2.88% of erythrocytes). We conclude that the high-dose anemia was a TNG-induced toxic effect.

Laboratory data from mice fed TNG for 12 or 24 months and allowed to recover for 1 month are shown in Tables 77 through 79. No males survived after 24 months. The anemia with sequelae which occurred in some high-dose mice fed TNG for 12 or 24 months was not seen in these mice after they were allowed to recover for 1 month. One of the geriatric control mice (No. 80-211) had profound anemia (3.46×10^6 erythrocytes per mm^3) and reticulocytosis (24.20%) with marked polychromasia and many target cells (Table 79).

E. Pathology

Data are included for 22 male and 37 female control mice, 32 and 37 low-dose mice, 18 and 30 middle-dose mice and 15 and 24 high-dose mice. Data are missing because of autolysis, cannibalism, etc.

1. Feeding for 12 Months

a. Organ Weights

Average organ weights of mice fed TNG for 12 months are given in Table 80. Unscheduled deaths, some as late as the night before necropsy, decreased the intended numbers of mice in some groups. The only toxicologically important finding was the decreased total body weight of the high-dose females. More subtle effects might have been obscured by individual variations within groups. For instance, control females Nos. 80-201 and 80-204 had enlarged livers weighing 2.04 g and 4.31 g, respectively, and massively enlarged spleens weighing 1.45 g and 1.32 g, respectively.

The findings in mice allowed to recover for 1 month are similar (Table 81). Although the total body weight of high-dose females was not decreased significantly, the relative brain weight was significantly increased.

b. Tissue Lesions

The lesions in mice fed TNG for 12 months are summarized in Tables 82 and 83. There were pigmentation and a hepatocellular dysplasia in most high-dose mice and a few others. The pigmentation was red to brown (often golden-brown) intracellular granules in the liver, spleen, and/or kidney. This pigment could be differentiated from normal hemosiderin deposits by its weak Prussian blue reaction, indicating low iron content. Hepatocellular dysplasia was characterized by polyploidy, inclusion bodies, or both, in the hepatocytes. Both lesions were related to TNG feeding. In addition, a variety of spontaneous lesions were seen in all mice. Most striking was the widespread presence of amyloid deposits, especially in the intestine and kidney. This amyloidosis is indicative of changes in old age.

If the mice were allowed to recover for 1 month after the 12 months' feeding, the lesions were similar (see Tables 84 and 85). In addition, the high-dose males had somewhat more severe hepatocellular dysplasia.

2. Feeding for 24 Months Including Unscheduled Deaths

a. Organ Weights

The organ weights from mice fed TNG for 24 months are shown in Table 86. There were no apparent changes in various organ weights. Organ weights in mice allowed to recover for 1 month (Table 87) are similar. Only females survived for this period. The ovary weight for one of two control mice (No. 80-221) was 11.40 g, 26% of total body weight, due to the presence of cystic ovaries.

b. Tissue Lesions

Lesions from mice fed TNG for 24 months are summarized in Tables 88 through 91. Lesions from mice dying at unscheduled times are summarized in Tables 92 through 97. The only effect apparently related to the TNG treatment was the incidence of deposits of a granular pigment, usually golden-brown, most commonly seen in the liver. This pigmentation occurred in most high-dose and some middle-dose mice. This hemosiderin-like pigment is presumably derived from hemoglobin degradation products. The minimal liver dysplasia seen after 12 months was now seen in all groups of mice. Lesions from mice allowed to recover for 1 month are summarized in Table 98. Pigmentation occurred in the liver and/or ovary of both high-dose females. No male mice survived during this period.

A large variety of spontaneous lesions were seen in these geriatric mice (Tables 88 through 98). The most common lesion was amyloid deposits in various visceral organs. In some cases, this was so widespread that it was merely referred to as "generalized amyloidosis." Other common lesions include a variety of minor, non-specific degenerative lesions, often labelled "aging changes." Some of the ovarian cysts, including that of No. 80-211, were actually hemorrhagic cysts outside the ovary, in the bursa ovarica, the peritoneal fossa in which the ovary lies. The occasional incidence of tumors is summarized in Table 99. None of these tumors is related to the TNG treatment.

F. Discussion

Although the mice consumed more TNG per unit body weight than the rats, they were less affected. The only toxicologically important effect was methemoglobinemia. This was seen in the high-dose mice only. This methemoglobinemia produced a toxic anemia, reflected by the presence of Heinz bodies in the blood and heme-derived pigment deposits in various organs, especially the liver. However, this anemia was mild and compensated for by the reserve capacity of the hematopoietic system; that is, its presence was inferred from the presence of reticulocytosis.

One other effect, a speeding up of the normal degenerative aging changes in the liver cells, as implied by the increased incidence of minimal hepatic dysplasia in high-dose mice killed after 12 months feeding, but not after 24 months feeding, may have occurred, However, the small numbers of animals killed after 12 months, and the mild, arguable presence of the lesions, make this effect questionable. Because no dose-related liver pathology (other than the granular pigment) was persistently seen in high-dose mice fed TNG for more than 12 months, we conclude that this liver effect was not real.

G. Conclusions

The low dose, with a TNG intake of about 11.1 mg/kg/day in males and 9.7 mg/kg/day in females, and the middle dose, with intakes of 115 and 96 mg/kg/day, respectively, were not toxic to mice. The high dose, about 1020 and 1060 mg/kg/day TNG intake for the males and females, respectively, was toxic. This dose produced lower feed consumption and weight gain, behavioral effects, and methemoglobinemia. The methemoglobinemia had sequelae including Heinz bodies, compensated anemia and pigment deposits.

TABLE 71

FEED CONSUMPTION AND COMPOUND INTAKE OF MICE FED TNG FOR 24 MONTHS

Dose (% in feed)	Males		Females	
	Feed Consumption (g/mouse/day)	TNG Intake (mg/kg/day)	Feed Consumption (g/mouse/day)	TNG Intake (mg/kg/day)
0	5.94 ± 0.17[a/]	--	4.32 ± 0.10	--
0.01	5.81 ± 0.20	11.10 ± 0.40	4.56 ± 0.09	9.72 ± 0.29
0.1	5.81 ± 0.21	114.6 ± 4.6	4.37 ± 0.12	96.4 ± 3.3
1	4.99 ± 0.18[b/]	1022 ± 38[b/]	4.10 ± 0.10	1058 ± 31

a/ Mean ± standard error of 24 measurements; the first month is the average of four measurements.
b/ Due to unscheduled deaths, only 23 monthly measurements.

TABLE 72

LABORATORY DATA OF MICE FED TNG AND DYING AT UNSCHEDULED TIMES

Dose (% in feed):	1	1	0	0.01	0	1	0.01	0.01	0.01	0.01	0.1	1
Mouse No.:	721	740	130	323	116	747	311	340	357	429	546	844
Week of Death:	38	53	62	62	62	62	78	78	78	78	78	78
Erythrocytes, x 10^6/mm^3	5.07	2.96	5.29	9.07	6.65	5.35	6.21	6.58	5.68	5.26	6.39	4.78
Heinz bodies, %	3.73	--	0.00	0.00	0.00	1.62	0.00	0.07	0.00	0.00	0.04	2.33
Reticulocytes, %	7.66	7.08	2.10	1.46	0.19	1.63	0.89	0.47	0.66	0.59	3.62	2.48
Hematocrit, vol. %	33	21	34	56	34	26	39	37	31	32	42	34
Hemoglobin, g %	9.8	6.3	10.9	17.7	11.2	9.3	11.0	11.5	10.1	10.1	12.4	10.8
Methemoglobin, %	4.1	--	3.4	--	0.0	0.0	0.0	0.0	5.0	0.0	2.4	4.6
MCV, cubic microns	65.1	70.9	64.3	61.7	51.1	48.6	62.8	56.2	61.0	60.8	65.7	71.1
MCHB, picograms	19.3	21.3	20.6	19.5	16.8	17.4	17.7	17.5	19.9	19.2	19.4	22.6
MCHB, g %	29.7	30.0	32.1	31.6	32.9	35.8	28.2	31.1	32.6	31.6	29.5	31.8
Platelets, x 10^5/mm^3	6.25	1.20	3.05	1.40	4.20	1.15	6.95	2.85	--	--	4.55	5.75
Leukocytes, x 10^3/mm^3	5.6	5.7	5.3[a]	10.9	2.0	1.0	8.1	1.5	2.9	6.3	4.1	2.5
Neutrophils, %	47	21	41	37	28	28	56	20	44	65	58	56
Lymphocytes, %	51	78	59	62	72	68	44	80	56	34	42	44
Bands, %	0	0	0	0	0	0	0	0	0	0	0	0
Monocytes, %	0	1	0	1	0	4	0	0	0	0	0	0
Eosinophils, %	2	0	0	0	0	0	0	0	0	1	0	0
Basophils, %	0	0	0	0	0	0	0	0	0	0	0	0
Atypical, %	0	0	0	0	0	0	0	0	0	0	0	0
Nucleated RBC, %	0	0	0	0	0	0	0	0	0	0	0	0
SGPT, IU/ℓ	28	55	24	31	--	49	34	62	--	24	--	52
BUN, mg/ℓ	21	62	24	165	--	--	41	27	--	17	--	28

TABLE 72 (Concluded)

Dose (% in feed):	0	0.01	0	0	1	0.1	0.01	1	0.1	1
Mouse No.:	226	339	258	259	822	631	436	852	629	837
Week of Death:	84	84	84	91	92	96	97	97	101	106[b]
Erythrocytes, x 10^6/mm^3	6.31	4.57	4.88	3.96	8.24	7.83	3.99	2.58	4.44	6.98
Heinz bodies, %	0.00	--	0.00	0.00	1.44	0.00	0.00	0.00	0.00	0.00
Reticulocytes, %	0.67	0.67	5.82	29.56	2.98	1.63	4.33	2.69	2.48	2.43
Hematocrit, vol. %	37	24	28	29	47	43	24	14	26	43
Hemoglobin, g %	11.8	8.4	9.1	8.8	15.3	15.2	8.4	5.3	9.4	13.9
Methemoglobin, %	0.0	--	0.0	7.8	7.5	0.0	3.6	5.6	8.2	0.0
MCV cubic microns	58.6	52.5	57.4	73.2	57.0	54.9	60.2	54.3	58.6	61.6
MCHB, picograms	18.7	18.4	18.6	22.2	18.6	19.4	21.1	20.5	21.2	19.9
MCHB, g %	31.9	35.0	32.5	30.3	32.6	35.3	35.0	37.9	36.2	32.3
Platelets, x 10^5/mm^3	7.05	2.35	3.05	2.15	4.90	5.20	5.65	2.65	--	2.65
Leukocytes, x 10^3/mm^3	5.0	3.6	6.2	17.6	5.2	3.5	3.0	3.0	9.9	8.7
Neutrophils, %	15	60	18	41	24	16	74	63	38	64
Lymphocytes, %	85	40	82	58	76	84	26	37	62	36
Bands, %	0	0	0	0	0	0	0	0	0	0
Monocytes, %	0	0	0	1	0	0	0	0	0	0
Eosinophils, %	0	0	0	0	0	0	0	0	0	0
Basophils, %	0	0	0	0	0	0	0	0	0	0
Atypical, %	0	0	0	0	0	0	0	0	0	0
Nucleated RBC, %	0	0	0	0	0	0	0	0	0	0
SGPT, IU/ℓ	93	--	121	34	--	37	52	99	--	238
BUN, mg/ℓ	31	--	80	62	100	19	33	82	78	29

a/ Occasional Howell-Lolly body seen.

b/ Fed TNG for 24 months; moribund during 2nd week of recovery.

TABLE 73

LABORATORY DATA OF MALE MICE AFTER FEEDING TNG FOR 12 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE (% IN FEED):	0 (C. 3)	0.01 (T. 4)	0.1 (T. 3)	1 (T. 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	7.57 ± .28	7.12 ± .31	8.11 ± .35	7.63 ± .28
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	.06 ± .06	2.41 ± .12[a/]
RETICULOCYTES, %	1.54 ± .11	1.74 ± .24	2.04 ± .40	2.78 ± .66
HEMATOCRIT, VOL. %	43.0 ± 1.0 (2)	41.3 ± 1.4	46.3 ± .9	45.5 ± .9
HEMOGLOBIN, GM. %	13.5 ± .4	13.0 ± .3	14.8 ± .3[a/]	14.2 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	.8 ± .4	2.3 ± .5[a/]	2.8 ± .0[a/]
MCV, CUBIC MICRONS	55.2 ± .7 (2)	58.0 ± 1.2	57.3 ± 1.6	59.8 ± 1.4
MCHH, MICRO MICROGMS.	17.8 ± .2	18.3 ± .4	16.3 ± .4	18.7 ± .6
MCHBC, GM %	32.0 ± .3 (2)	31.5 ± .4	31.9 ± .4	31.3 ± .6
PLATELETS ($\times 10^5$ /MM^3)	2.7 ± .7	4.6 ± .9	3.2 ± .0 (2)	5.7 ± 1.0
LEUKOCYTES ($\times 10^3$ /MM^3)	4.4 ± 1.4	2.3 ± .4	4.4 ± 1.9	5.7 ± .6
NEUTHOPHILS, %	30.0 ± 2.5	18.3 ± 1.7	29.3 ± 4.7	21.8 ± 4.1
LYMPHOCYTES, %	69.3 ± 2.8	80.3 ± 2.1	70.0 ± 4.6	77.5 ± 4.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	.5 ± .5	.3 ± .3	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.7 ± .3	1.0 ± 1.0	.3 ± .3	.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGPT, IU/L	21.0 (1)	22.3 ± 7.5 (3)	38.0 ± 5.6	42.0 ± 2.6 (3)
BUN, MG %	22.0 (1)	30.3 ± 5.9 (3)	26.3 ± 3.5	22.3 ± 1.7 (3)

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 74

LABORATORY DATA OF FEMALE MICE AFTER FEEDING TNG FOR 12 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE (% IN FEED):	0 (C. 4)	0.01 (T. 4)	0.1 (T. 4)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.39 ± .66	7.52 ± .24	7.29 ± .15	7.38 ± .32
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	.93 ± .12[a/]
RETICULOCYTES, %	3.13 ± 1.57	1.22 ± .26	2.18 ± .43	2.49 ± .38
HEMATOCRIT, VOL. %	42.3 ± 2.7	43.3 ± 1.0	43.3 ± .7 (3)	43.5 ± .6
HEMOGLOBIN, GM. %	13.6 ± 1.1	14.0 ± .5	13.9 ± .3	13.5 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	57.7 ± 2.1	57.6 ± .7	59.8 ± 1.1 (3)	59.1 ± 2.0
MCHB, MICRO MICROGMS.	18.5 ± .2	18.6 ± .3	19.0 ± .1	18.3 ± .1
MCHBC, GM %	32.2 ± .8	32.3 ± .5	31.9 ± .5 (3)	30.9 ± .9
PLATELETS ($X10^5$ /MM^3)	3.0 (1)	3.2 ± .8 (3)	3.5 (1)	6.3 ± 2.1 (2)
LEUKOCYTES ($X10^3$ /MM^3)	6.3 ± 2.3	1.9 ± .5	4.4 ± 1.4	3.2 ± .6
NEUTROPHILS, %	16.7 ± 1.3 (3)	16.8 ± 2.1	26.0 ± 7.0	14.0 ± 2.0
LYMPHOCYTES, %	79.7 ± 3.3 (3)	82.3 ± 2.7	73.3 ± 6.9	85.5 ± 2.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.8 ± 1.7	1.0 ± .7	.5 ± .5	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGPT, IU/L	38.3 ± 14.2	31.0 ± 2.1	29.7 ± 3.0 (3)	37.5 ± 4.7
BUN, MG %	35.3 ± 8.1	26.5 ± 2.3	22.[illegible] ± .7 (3)	27.8 ± 1.8

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 75

LABORATORY DATA OF MALE MICE AFTER FEEDING TNG FOR 24 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE (% IN FEED):	0.0 (C, 1)	0.01 (T, 2)	0.1 (T, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	6.76	8.12 ± .12	4.97 ± .34
HEINZ BODIES, %	0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	2.01	2.12 ± .08	3.21 ± 1.27
HEMATOCRIT, VOL. %	41.0	44.5 ± 2.5	33.0 ± 3.0
HEMOGLOBIN, GM. %	13.2	15.2 ± .4	11.4 ± 1.1
METHEMOGLOBIN, %	3.6	.8 ± .8	2.3 ± 2.3
MCV, CUBIC MICRONS	60.7	54.8 ± 2.3	67.2 ± 10.7
MCHB, MICRO MICROGMS.	19.5	18.7 ± .2	23.2 ± 3.8
MCHBC, GM %	32.2	34.2 ± 1.0	34.5 ± .2
PLATELETS ($X10^5$ /MM^3)	4.8	4.7 ± .5	5.2 ± 1.6
LEUKOCYTES ($X10^3$ /MM^3)	1.5	3.8 ± 1.1	1.3 ± .1
NEUTROPHILS, %	54.0	31.0 ± 7.0	49.0 ± 5.0
LYMPHOCYTES, %	46.0	66.5 ± 4.5	51.0 ± 5.0
BANDS, %	0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0	1.5 ± 1.5	0.0 ± 0.0
BASOPHILS, %	0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0	1.0 ± 1.0	0.0 ± 0.0
ATYPICAL, %	0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0	0.0 ± 0.0	0.0 ± 0.0
SGOT, IU/L	77	84 ± 22	532 ± 213
SGPT, IU/L	34	47 ± 13	342 ± 209
BUN, MG %	50.0	27.0 ± 2.0	42.5 ± 3.5

ENTRIES ARE MEAN ± STANDARD ERROR

TABLE 76

LABORATORY DATA OF FEMALE MICE AFTER FEEDING TNG FOR 24 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF MICE

DOSE (% IN FEED):	0 (C, 4)	0.01 (T, 4)	0.1 (T, 4)	1 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.30 ± .30	6.02 ± .60	4.49 ± .77	5.29 ± .43
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	.1 ± .1	1.9 ± .4[a/]
RETICULOCYTES, %	1.34 ± .31	2.43 ± .22	8.50 ± 2.15[a/]	2.51 ± .66
HEMATOCRIT, VOL. %	36.8 ± 2.1	35.8 ± 3.7	30.8 ± 4.5	37.3 ± 1.6
HEMOGLOBIN, GM. %	12.9 ± .8	12.2 ± 1.2	10.7 ± 1.4	12.9 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.9 ± .9	1.8 ± .8
MCV, CUBIC MICRONS	58.3 ± 1.0	59.3 ± 2.5	70.5 ± 7.2	72.6 ± 9.3
MCHB, MICRO MICROGMS.	20.4 ± .5	20.3 ± .9	24.8 ± 2.7	25.1 ± 3.1
MCHBC, GM %	35.3 ±	34.3 ± .5	35.2 ± .6	34.7 ± .3
PLATELETS ($X10^5$ /MM^3)	3.7 ± .6	4.7 ± .6 (3)	4.7 ± .5	4.3 ± .1 (3)
LEUKOCYTES ($X10^3$ /MM^3)	2.5 ± .6	3.6 ± .7	3.8 ± 1.9	1.7 ± .4
NEUTROPHILS, %	45.3 ± 5.9	32.8 ± 5.8	44.5 ± 10.2	29.0 ± 5.9
LYMPHOCYTES, %	54.3 ± 5.8	65.5 ± 5.2	54.5 ± 10.4	68.8 ± 5.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	.8 ± .5	.8 ± .8	1.5 ± .6
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	1.0 ± .6	.3 ± .3	.8 ± .8
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGOT, IU/L	148 ± 19	156 ± 60	139 ± 19	95 ± 10 (3)
SGPT, IU/L	40.8 ± 3.8	82.5 ± 50.2	30.5 ± 6.6	39.3 ± 11.3 (3)
BUN, MG %	32.5 ± 6.2	24.5 ± 5.6	15.8 ± 4.2	23.3 ± 4.9 (3)

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 77

LABORATORY DATA OF MALE MICE AFTER FEEDING TNG FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE (% IN FEED):	0 (C. 3)	0.01 (T. 4)	0.1 (T. 4)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.91 ± .45	8.14 ± .01	7.40 ± .23	7.83 ± .29 (3)
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	.20 ± .12
RETICULOCYTES, %	.93 ± .09	1.09 ± .16	1.13 ± .14	1.21 ± .12 (3)
HEMATOCRIT, VOL. %	44.7 ± 2.6	46.8 ± .5	42.8 ± 1.7	45.0 ± 1.2 (3)
HEMOGLOBIN, GM. %	12.1 (1)	14.7 ± .1	13.2 ± .4	14.3 ± .7 (3)
METHEMOGLOBIN, %	0.0 ± 0.0	.7 ± .7	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	56.5 ± .4	57.4 ± .6	57.7 ± 1.5	57.5 ± .9 (3)
MCHB, MICRO MICROGMS.	16.9 (1)	18.0 ± .1	17.8 ± .6	18.2 ± .3 (3)
MCHBC, GM %	30.3 (1)	31.4 ± .3	30.8 ± .3	31.9 ± 1.0 (3)
PLATELETS ($X10^5$ /MM^3)	7.9 ± .6	5.5 ± .9	7.0 ± .6	8.3 ± 2.1
LEUKOCYTES ($X10^3$ /MM^3)	3.8 ± .5	4.0 ± .7	3.6 ± .4	3.7 ± .4 (3)
NEUTROPHILS, %	27.3 ± 9.2	49.5 ± 3.0	32.3 ± 6.7	23.0 ± 5.1
LYMPHOCYTES, %	45.3 ± 21.6	50.0 ± 2.9	66.5 ± 6.2	73.8 ± 5.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.5 ± .5
EOSINOPHILS, %	27.3 ± 27.3	.5 ± .3	1.3 ± .6	2.8 ± 1.1
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGPT, IU/L	24.3 ± 2.0	31.0 ± 1.2	29.8 ± 4.9	41.0 ± 2.6 (3)[a/]
BUN, MG %	28.7 ± 2.4	28.3 ± 2.9	30.5 ± 4.1	22.0 ± .6 (3)

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 78

LABORATORY DATA OF FEMALE MICE AFTER FEEDING TNG FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE (% IN FEED):	0 (C. 4)	0.01 (T. 4)	0.1 (T. 3)	1 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.42 ± .34	7.57 ± .46	7.00 ± .29	7.25 ± .12
HEINZ BODIES, %	.02 ± .02	0.00 ± 0.00	0.00 ± 0.00	.05 ± .05
RETICULOCYTES, %	.97 ± .23	1.04 ± .05	1.78 ± .52	1.81 ± .38
HEMATOCRIT, VOL. %	42.8 ± 2.6	43.3 ± 1.7	42.7 ± 1.8	44.0 ± .7
HEMOGLOBIN, GM. %	13.4 ± .6	13.5 ± .9	13.5 ± .6	14.1 ± .1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	57.5 ± 1.4	57.3 ± 1.3	61.0 ± .8	60.7 ± .3
MCHH, MICRO MICROGMS.	18.1 ± .1	17.9 ± .2	19.2 ± .2[a]	19.5 ± .3[a]
MCHBC, GM %	31.5 ± .6	31.2 ± .9	31.6 ± .8	32.2 ± .4
PLATELETS ($X10^5$ /MM^3)	5.8 ± .8	6.6 ± 1.5	5.9 ± 2.0	5.4 ± 1.2 (3)
LEUKOCYTES ($X10^3$ /MM^3)	3.5 ± .8	2.5 ± .3	3.0 ± .3	4.6 ± 1.0
NEUTROPHILS, %	23.8 ± 7.4	25.5 ± 5.2	23.0 ± 4.6	24.5 ± 2.9
LYMPHOCYTES, %	75.0 ± 6.9	74.0 ± 4.7	75.3 ± 5.6	71.5 ± 3.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± .4[a]
EOSINOPHILS, %	.5 ± .5	.5 ± .5	1.7 ± 1.2	1.5 ± .6
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .5	0.0 ± 0.0	0.0 ± 0.0	1.5 ± .9
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGPT, IU/L	37.0 ± 3.2	32.3 ± 4.8	32.0 ± 2.0	26.3 ± 2.8
BUN, MG %	46.5 ± 13.3	26.5 ± 3.9	19.7 ± 2.3	21.5 ± 2.5

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 79

LABORATORY DATA OF FEMALE MICE AFTER FEEDING TNG FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C,N) CONTROL (T,N) TREATED N = NUMBER OF MICE

DOSE (% IN FEED):	0 (C, 2)	0.01 (T, 2)	0.1 (T, 2)	1 (T, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	4.71 ± 1.26	5.91 ± .25	6.58 ± 1.45	5.63 ± .38
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	12.50 ± 11.69	.93 ± .03	2.69 ± 1.50	1.18 ± .82
HEMATOCRIT, VOL. %	32.5 ± 5.5	38.0 ± 2.0	38.0 ± 5.0	40.0 ± 2.0
HEMOGLOBIN, GM. %	10.9 ± 1.7	12.6 ± .8	13.4 ± 2.2	12.2 ± 1.2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	70.8 ± 7.2	64.2 ± .6	58.9 ± 5.4	71.2 ± 1.2
MCHB, MICRO MICROGMS.	23.9 ± 2.7	21.3 ± .4	20.7 ± 1.3	21.6 ± .7
MCHBC, GM %	33.7 ± .3	33.1 ± .4	35.3 ± 1.0	30.4 ± 1.5
PLATELETS ($X10^5$ /MM^3)	7.5 ± .7	18.6 ± 10.6	4.5 ± .4	5.8 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	2.8 ± 2.0	2.6 ± 1.1	5.9 ± 2.7	4.1 ± 1.5
NEUTROPHILS, %	26.5 ± .5	34.0 ± 4.0	30.5 ± 23.5	12.5 ± .5
LYMPHOCYTES, %	72.5 ± .5	64.5 ± 2.5	69.5 ± 23.5	86.0 ± 1.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	.5 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGOT, IU/L	107 ± 27	112 ± 13	236 ± 140	137 ± 47
SGPT, IU/L	37.0 ± 6.0	38.5 ± 7.5	52.5 ± 12.5	37.0 (1)
BUN, MG %	38.0 ± 6.0	55.0 ± 29.0	39.0 ± 0.0	32.0 ± 8.0

ENTRIES ARE MEAN ± STANDARD ERROR

TABLE 80

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED TMG FOR 12 MONTHS

Sex	Dose (% in feed)	Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male		4[illegible] [a]	0.49 ± 0.02	0.18 ± 0.00	1.74 ± 0.07	0.63 ± 0.01	0.12 ± 0.01	0.25 ± 0.04	
	.01	45 ± 2 [b]	0.47 ± 0.01	0.20 ± 0.01	1.91 ± 0.13	0.73 ± 0.06	0.12 ± 0.02	0.24 ± 0.01	
	0.1	44 ± 2 [a]	0.49 ± 0.01	0.20 ± 0.02	1.89 ± 0.18	0.77 ± 0.11	0.13 ± 0.02	0.24 ± 0.03	
	1	42 ± 1 [b]	0.45 ± 0.01	0.17 ± 0.01	1.94 ± 0.07	0.61 ± 0.05	0.18 ± 0.01	0.25 ± 0.01	
Female	0	40 ± 1 [b]	0.50 ± 0.01	0.14 ± 0.01	2.25 ± 0.71	0.43 ± 0.01	0.75 ± 0.37		0.048 ± 0.019
	0.01	37 ± 1 [b]	0.50 ± 0.02	0.15 ± 0.01	1.37 ± 0.04	0.46 ± 0.02	0.11 ± 0.01		0.042 ± 0.008
	0.1	40 ± 2 [b]	0.51 ± 0.01	0.14 ± 0.02	1.50 ± 0.16	0.42 ± 0.03	0.15 ± 0.02		0.061 ± 0.023
	1	32 ± 1 [b,c]	0.47 ± 0.01	0.12 ± 0.01	1.44 ± 0.05	0.38 ± 0.02	0.17 ± 0.03		0.037 ± 0.011

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	1.12 ± 0.05	0.41 ± 0.01	4.01 ± 0.19	1.45 ± 0.03	0.29 ± 0.03	0.58 ± 0.10	
	0.01	1.05 ± 0.05	0.44 ± 0.01	4.26 ± 0.14	1.62 ± 0.08	0.27 ± 0.03	0.54 ± 0.03	
	0.1	1.13 ± 0.06	0.46 ± 0.01	4.32 ± 0.40	1.78 ± 0.29	0.31 ± 0.05	0.55 ± 0.07	
	1	1.06 ± 0.03	0.40 ± 0.03	4.60 ± 0.23	1.45 ± 0.11	0.41 ± 0.03	0.59 ± 0.03	
Female	0	1.27 ± 0.03	0.36 ± 0.04	5.60 ± 1.56	1.10 ± 0.05	1.87 ± 0.92		0.124 ± 0.051
	0.01	1.37 ± 0.05	0.41 ± 0.03	3.72 ± 0.07	1.25 ± 0.06	0.30 ± 0.03		0.115 ± 0.020
	0.1	1.28 ± 0.08	0.35 ± 0.04	3.73 ± 0.34	1.06 ± 0.06	0.36 ± 0.04		0.156 ± 0.062
	1	1.46 ± 0.05	0.38 ± 0.02	4.50 ± 0.21	1.18 ± 0.07	0.53 ± 0.08		0.116 ± 0.033

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					
		Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.36 ± 0.02	3.57 ± 0.12	1.29 ± 0.04	0.25 ± 0.02	0.53 ± 0.11	
	0.01	0.42 ± 0.02	4.07 ± 0.26	1.55 ± 0.12	0.26 ± 0.03	0.52 ± 0.02	
	0.1	0.41 ± 0.04	3.84 ± 0.41	1.57 ± 0.23	0.27 ± 0.03	0.49 ± 0.06	
	1	0.38 ± 0.02	4.33 ± 0.10	1.37 ± 0.11	0.39 ± 0.02 [c]	0.55 ± 0.03	
Female	0	0.28 ± 0.03	4.48 ± 1.38	0.86 ± 0.03	1.50 ± 0.74		0.096 ± 0.037
	0.01	0.30 ± 0.02	2.72 ± 0.10	0.91 ± 0.05	0.23 ± 0.03		0.086 ± 0.018
	0.1	0.27 ± 0.03	2.94 ± 0.29	0.83 ± 0.05	0.29 ± 0.04		0.119 ± 0.043
	1	0.26 ± 0.02	3.09 ± 0.12	0.81 ± 0.03	0.36 ± 0.05		0.082 ± 0.025

a/ Mean ± standard error of three mice.
b/ Mean ± standard error of four mice.
c/ Significantly different from control mice by Dunnett's multiple comparison procedure.

TABLE 81

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED TNG FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	43 ± 2[a]	0.49 ± 0.02	0.23 ± 0.01	1.90 ± 0.10	0.77 ± 0.05	0.15 ± 0.02	0.24 ± 0.05	
	0.01	43 ± 2[b]	0.48 ± 0.01	0.22 ± 0.01	1.88 ± 0.16	0.77 ± 0.04	0.14 ± 0.01	0.26 ± 0.03	
	0.1	43 ± 2[b]	0.49 ± 0.01	0.21 ± 0.02	2.06 ± 0.13	0.65 ± 0.01	0.16 ± 0.02	0.30 ± 0.02	
	1	37 ± 1[b]	0.45 ± 0.01	0.21 ± 0.04	1.74 ± 0.13	0.60 ± 0.05[c]	0.17 ± 0.03	0.21 ± 0.01	
Female	0	34 ± 1[b]	0.48 ± 0.01	0.15 ± 0.01	1.58 ± 0.16	0.48 ± 0.05	0.33 ± 0.16		0.091 ± 0.020
	0.01	38 ± 2[b]	0.48 ± 0.01	0.15 ± 0.01	1.55 ± 0.12	0.47 ± 0.05	0.74 ± 0.47		0.039 ± 0.004
	0.1	34 ± 1[a]	0.49 ± 0.01	0.16 ± 0.00	1.31 ± 0.08	0.43 ± 0.01	0.11 ± 0.02		0.042 ± 0.008
	1	29 ± 1[b]	0.50 ± 0.02	0.16 ± 0.01	1.33 ± 0.08	0.41 ± 0.01	0.13 ± 0.01		0.082 ± 0.040

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	1.15 ± 0.09	0.55 ± 0.05	4.43 ± 0.09	1.80 ± 0.03	0.34 ± 0.02	0.56 ± 0.09	
	0.01	1.12 ± 0.05	0.51 ± 0.02	4.33 ± 0.22	1.78 ± 0.08	0.33 ± 0.02	0.61 ± 0.08	
	0.1	1.13 ± 0.03	0.49 ± 0.01	4.81 ± 0.44	1.51 ± 0.05	0.37 ± 0.05	0.71 ± 0.07	
	1	1.23 ± 0.03	0.56 ± 0.08	4.73 ± 0.17	1.65 ± 0.11	0.46 ± 0.07	0.59 ± 0.02	
Female	0	1.39 ± 0.06	0.44 ± 0.02	4.58 ± 0.35	1.40 ± 0.16	0.92 ± 0.40		0.269 ± 0.067
	0.01	1.28 ± 0.06	0.39 ± 0.03	4.09 ± 0.21	1.24 ± 0.07	2.04 ± 1.32		0.102 ± 0.012
	0.1	1.44 ± 0.09	0.48 ± 0.03	3.86 ± 0.43	1.26 ± 0.11	0.34 ± 0.09		0.125 ± 0.028
	1	1.70 ± 0.06[c]	0.55 ± 0.03	4.53 ± 0.26	1.39 ± 0.05	0.43 ± 0.03		0.284 ± 0.144

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					
		Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.48 ± 0.01	3.89 ± 0.29	1.59 ± 0.14	0.30 ± 0.04	0.50 ± 0.11	
	0.01	0.46 ± 0.01	3.91 ± 0.35	1.60 ± 0.09	0.30 ± 0.02	0.54 ± 0.06	
	0.1	0.43 ± 0.02	4.24 ± 0.35	1.33 ± 0.02	0.33 ± 0.05	0.63 ± 0.05	
	1	0.46 ± 0.08	3.87 ± 0.21	1.35 ± 0.11	0.38 ± 0.07	0.48 ± 0.02	
Female	0	0.32 ± 0.02	3.33 ± 0.36	1.01 ± 0.10	0.70 ± 0.34		0.191 ± 0.042
	0.01	0.30 ± 0.02	3.21 ± 0.22	0.97 ± 0.09	1.56 ± 0.01		0.080 ± 0.009
	0.1	0.33 ± 0.01	2.67 ± 0.15	0.87 ± 0.03	0.23 ± 0.05		0.085 ± 0.016
	1	0.33 ± 0.02	2.69 ± 0.22	0.83 ± 0.04	0.26 ± 0.29		0.164 ± 0.081

a/ Mean ± standard error of three mice.
b/ Mean ± standard error of four mice.
c/ Significantly different from control mice by Dunnett's multiple comparison procedure.

TABLE 82

SUMMARY OF LESIONS IN MALE MICE FED TNG FOR 12 MONTHS

Dose (% in feed):	0			0.01				0.1			1			
Mouse No.:	101	103	104	301	302	303	304	502	503	504	701	702	703	704
Treatment-Related Lesions[a]														
Liver														
Pigmentation											1	1	2	2
Hepatocellular dysplasia				1				1		1	1	1	1	1
Other Lesions														
Adrenal Gland														
Amyloidosis				3	2	2			2	3	3		1	
Fibroblast proliferation	2				1	3	1		1	1		1		1
Ceroid degeneration	2	1		1			1		1					
Tyroid														
Tyroiditis					1									
Lung														
Peribronchiolar lymphoid proliferation	1	1	1		1			1	1			1	1	1
Bronchiectasis with/without emphysema		1												
Heart														
Amyloidosis		1							1		1		1	
Liver														
Amyloidosis		1			1						1		2	
Portal inflammation		1	1		1	1	1	1						
Testis														
Amyloidosis				4			1			1				
Degeneration with/without atrophy		2		4						1		±		
Pancreas														
Mononuclear cell foci													1	
Salivary Gland														
Mononuclear cell foci					1									
Stomach														
Amyloidosis								1						
Focal calcification		1						1						
Gastritis or ulceration				1									1	
Intestine														
Amyloidosis			3		1	1			1	1	3	2		2
Parasitism							1							
Lymphoid hyperplasia		1									1			
Kidney														
Amyloidosis				3	3	3	1			3	2		1	1
Perivascular cuffings	1		1	1	1	1	1	1		1	2	1	1	1
Nephrosis (focal)	1													
Eye														
Retinal atrophy		1	1			2	1			1	1			1
Cataract	1	1												
Lymph Node														
Lymphoid hyperplasia		1												
Bone Marrow M/E	1.3	2.0	1.0	1.3	1.2	1.6	2.2	2.2	1.9	2.2	1.7	1.5	1.0	1.8

Tissues not listed were normal.

a/ Severity of lesions: 1 - mild; 2 - moderate, 3 - marked, 4 - severe, ± - minimal or questionable; X - present; 0 - tissue missing or unreadable.

TABLE 83

SUMMARY OF LESIONS IN FEMALE MICE FED TNG FOR 12 MONTHS

Dose (% in feed):	0				0.01				0.1				1			
Mouse No.:	201	202	203	204	401	402	403	404	601	602	603	604	801	802	803	804
Treatment-Related Lesions[a]																
Liver																
Pigmentation													1		1	
Hepatocellulary dysplasia	1															1
Spleen																
Excessive pigmentation													1		1	1
Kidney																
Pigmentation													3			3
Other Lesions																
Adrenal Gland																
Amyloidosis										2			3	1	3	1
Fibroblast proliferation	1		1		1	1	1	1	1		1	1	2		1	1
Ceroid degeneration	1															
Trachea																
Tracheitis	1															
Thyroid																
Amyloidosis										1			1		1	
Tyroiditis			1													
Lung																
Peribronchiolar lymphoid proliferation			1		1	1	1	1		1	1	1	1	2	1	
Amyloidosis													1			
Bronchiectasis with/without emphysema			1													
Reticuloendotheliosis	2															
Heart																
Amyloidosis										1			1			
Liver																
Amyloidosis													2			
Portal inflammation			1			1		1	1	1			1	1	1	
Reticuloendotheliosis	2															
Hyperplastic foci		1														
Microscopic granuloma		1				1				1		1				
Fibrosarcoma			X													
Spleen																
Reticuloendotheliosis	3															
Excessive extramedullary hematopoiesis			2													
Fibrosarcoma			X													
Ovary																
Amyloidosis			1										2		1	
Ovarian cyst		1	1													
Uterus																
Endometrial hyperplasia	2			1	1	2	1		1			1				
Amyloidosis										1					1	
Salivary Gland																
Mononuclear cell foci	1	1	1									1				
Stomach																
Amyloidosis										1						
Gastritis or ulceration																1
Intestine																
Amyloidosis	3		1	1	1			2	1	4	1	2	1	1		1
Parasitism												1				
Lymphoid hyperplasia	1															
Kidney																
Amyloidosis										3	1	1	3		1	
Perivascular cuffings	1	1	1	1	2	1	2	1	1	1		3	1	1	1	2
Nephrosis (focal)							1									
Hydronephrosis			4													
Eye																
Retinal atrophy	1		1	1		1								1		
Lymph Node																
Lymphoid hyperplasia			1													
Fibrosarcoma				X												
Urinary Bladder																
Mononuclear cell foci			1												1	
Skin																
Subcutaneous edema				1												
Bone Marrow M/E	1.7	1.5	1.7	5.9	1.1	1.3	1.6	1.5	1.8	2.3	1.5	2.4	1.3	1.2	1.0	1.1

Tissues not listed were normal.

a/ Severity of lesions: 1 - mild; 2 - moderate, 3 - marked; 4 - severe; ± - minimal or questionable; X - present; 0 - tissue missing or unreadable.

TABLE 84

SUMMARY OF LESIONS IN MALE MICE FED TNG FOR 17 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0			0.01				0.1				1			
Mouse No.:	105	107	108	305	306	307	308	505	506	507	508	705	706	707	708
Treatment-Related Lesions[a]															
Liver															
Pigmentation												3	1	3	1
Hepatocellular dysplasia		1		1	1	1				1	1	2	2	2	1
Spleen															
Excessive pigmentation														2	
Other Lesions															
Adrenal Gland															
Amyloidosis	3	2								3	1		3		
Fibroblast proliferation	1			1		1		1		1		1		1	1
Cystoid degeneration			1	1		1	1	1				1			
Parathyroid															
Amyloidosis															1
Thyroid															
Amyloidosis	1												2		
Thyroiditis														1	
Lung															
Peribronchiolar lymphoid proliferation			1			1		1		1		1	1	1	2
Focal pleuritis											1	1			
Heart															
Amyloidosis	1	1	1		1			1	1	1	1		2		
Liver															
Portal inflammation	1									1	1	1	1	1	
Amyloidosis	1														
Hepatoma									X						
Microabscess												1			
Spleen															
Lymphoid hyperplasia								1							
Testis															
Amyloidosis	3		1					1							
Degeneration and atrophy	1		1				1								±
Pancreas															
Mononuclear cells foci		1													
Salivary Gland															
Mononuclear cells foci					1		1	1				1			
Lymph Node															
Amyloidosis			1								1				
Stomach															
Amyloidosis	1												1		
Intestine															
Amyloidosis	3	3	3			2	1	3	3	2	3		3		
Parasitism	2	1	1												
Lymphoid hyperplasia									1						
Kidney															
Amyloidosis	3	3	2					3	1	2	3		3	1	2
Perivascular cuffings	1	1	1	1	1	1		1	1	1	2	1	1	1	±
Nephrosis					1										
Urinary Bladder															
Mononuclear cells foci					1								±		
Eye															
Retinal atrophy		1				1	2					1			
Skin															
Abscessation						1									
Bone Marrow															
M/E Ratio	1.8	1.0	1.5	2.1	1.4	2.0	1.3	1.4	1.0	1.5	1.0	2.1	1.6	1.7	1.2

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = marked, 4 = severe, ± = minimal or questionable, X = present, 0 = tissue missing or unreadable.

TABLE 85

SUMMARY OF LESIONS IN FEMALE MICE FED TNG FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0				0.01				0.1			1			
Mouse No.:	205	206	207	208	405	406	407	408	605	607	608	805	806	807	808
Treatment-Related Lesions[a]															
Liver															
Pigmentation												2		1	
Hepatocellular dysplasia	1			1								1		1	
Spleen															
Excessive pigmentation												1	1	1	1
Other Lesions															
Adrenal Gland															
Amyloidosis	1							1							
Fibroblast proliferation	1	1	1	1	1	1		1	1	1	1	2	1		
Trachea															
Tracheitis															
Thyroid															
Amyloidosis	1														
Thyroiditis		1	1	1											
Lung															
Peribronchiolar lymphoid proliferation		1	1	1	1	1	1	1		1	1	1	1		1
Heart															
Amyloidosis		1		1			1								
Liver															
Portal inflammation	1	1	1		2			1	1			1			
Necrosis			2												
Fatty toma						X									
Microabscess											1				1
Spleen															
Lymphoid hyperplasia	1				2	1									
Ovary															
Amyloidosis	3	3	3	3				3							
Ovarian cyst			1									1			
Uterus															
Endometrial hyperplasia				1		1	1	1	2	1		1			
Endometritis	1		2												2
Pancreas															
Mononuclear cells foci	2		1		1										
Salivary Gland															
Mononuclear cells foci	1	1	1	1	1			1			1	1			
Amyloidosis				1											
Stomach															
Amyloidosis	1	1													
Gastritis	1	1													
Intestine															
Amyloidosis	3	3	4	3			4	3							
Parasitism		1	1												
Lymphoid hyperplasia											1				
Kidney															
Amyloidosis	1	1	1					3							
Perivascular cuffings	1		2		2	2	1	2	2	1	1	2	1	2	1
Nephrosis		1			1							1	1		
Cystic kidney			4						1						
Nephritis				3							1				
Urinary Bladder															
Mononuclear cells foci	1		1			1									
Eye															
Retinal atrophy		1			1		1	1							
Conjunctivitis														1	
Bone Marrow															
M/E Ratio	2.0	1.2	2.0	2.1	1.2	1.5	1.0	1.4	1.0	1.0	2.0	1.0	1.0	1.0	1.0

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = marked, 4 = severe, ± = minimal or questionalbe, X = present, 0 = tissue missing or unreadable.

TABLE 86

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED TMC FOR 24 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	37[a]	0.43	0.25	1.77	0.65	0.10	0.20	
	0.01	41 ± 2[b]	0.47 ± 0.02	0.22 ± 0.02	1.95 ± 0.02	0.80 ± 0.02	0.38 ± 0.07	0.17 ± 0.03	
	0.1	34 ± 2[b]	0.42 ± 0.01	0.21 ± 0.02	1.87 ± 0.05	0.57 ± 0.02	0.18 ± 0.07	0.16 ± 0.01	
Female	0	37 ± 1[c]	0.49 ± 0.01	0.18 ± 0.01	1.66 ± 0.09	0.50 ± 0.03	0.18 ± 0.04		0.28 ± 0.08
	0.01	38 ± 2[d]	0.48 ± 0.02	0.18 ± 0.01	1.84 ± 0.11	0.60 ± 0.12	0.25 ± 0.06		0.45 ± 0.21
	0.1	35 ± 2[e]	0.49 ± 0.01	0.19 ± 0.01	1.84 ± 0.05	0.52 ± 0.02	0.24 ± 0.03		0.40 ± 0.15
	1	32 ± 1[f]	0.46 ± 0.01	0.20 ± 0.01	1.72 ± 0.12	0.50 ± 0.04	0.19 ± 0.04		0.35 ± 0.13

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	1.16	0.68	4.78	1.76	0.27	0.54	
	0.01	1.13 ± 0.02	0.52 ± 0.02	4.67 ± 0.28	1.92 ± 0.15	0.91 ± 0.21	0.42 ± 0.10	
	0.1	1.25 ± 0.06	0.62 ± 0.02	5.51 ± 0.18	1.70 ± 0.14	0.53 ± 0.19	0.47 ± 0.00	
Female	0	1.32 ± 0.05	0.49 ± 0.02	4.43 ± 0.20	1.34 ± 0.05	0.49 ± 0.10		0.70 ± 0.18
	0.01	1.30 ± 0.06	0.50 ± 0.02	5.00 ± 0.37	1.58 ± 0.29	0.63 ± 0.13		1.14 ± 0.54
	0.1	1.42 ± 0.08	0.56 ± 0.05	5.26 ± 0.13	1.48 ± 0.06	0.68 ± 0.07		1.20 ± 0.48
	1	1.45 ± 0.07	0.61 ± 0.03[g]	5.33 ± 0.28	1.54 ± 0.09	0.58 ± 0.10		1.06 ± 0.37

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					
		Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.58	4.12	1.51	0.23	0.47	
	0.01	0.46 ± 0.03	4.12 ± 0.18	1.70 ± 0.10	0.81 ± 0.17	0.37 ± 0.09	
	0.1	0.49 ± 0.04	4.47 ± 0.07	1.35 ± 0.05	0.43 ± 0.17	0.38 ± 0.02	
Female	0	0.38 ± 0.02	3.41 ± 0.20	1.03 ± 0.06	0.37 ± 0.07		0.57 ± 0.16
	0.01	0.39 ± 0.02	3.87 ± 0.24	1.31 ± 0.34	0.51 ± 0.12		0.90 ± 0.43
	0.1	0.40 ± 0.03	3.77 ± 0.15	1.06 ± 0.05	0.50 ± 0.08		0.82 ± 0.31
	1	0.43 ± 0.03	3.75 ± 0.32	1.08 ± 0.10	0.41 ± 0.08		0.77 ± 0.28

a/ One surviving mouse.
b/ Mean ± standard error of two mice.
c/ Mean ± standard error of 13 mice.
d/ Mean ± standard error of 12 mice.
e/ Mean ± standard error of eight mice.
f/ Mean ± standard error of six mice.
g/ Significantly different from control mice by Dunnett's multiple comparison procedure.

TABLE 87

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE MICE FED TNG FOR 24 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)					
		Brain	Heart	Liver	Kidney	Spleen	Ovary
0	44 ± 1[a]	0.48 ± 0.01	0.16 ± 0.00	1.56 ± 0.19	0.54 ± 0.05	0.28 ± 0.07	5.78 ± 5.62
0.01	40 ± 1[b]	0.50 ± 0.02	0.22 ± 0.02	2.00 ± 0.16	0.54 ± 0.04	0.21 ± 0.03	0.62 ± 0.56
0.1	37 ± 9[a]	0.45 ± 0.02	0.17 ± 0.01	2.70 ± 1.27	0.57 ± 0.16	0.43 ± 0.36	0.85 ± 0.82
1	31 ± 0[a]	0.47 ± 0.02	0.16 ± 0.01	1.51 ± 0.06	0.48 ± 0.02	0.12 ± 0.01	0.12 ± 0.02

Dose (% in feed)	Relative Organ Weight (g/100 g body weight)					
	Brain	Heart	Liver	Kidney	Spleen	Ovary
0	1.10 ± 0.01	0.37 ± 0.00	3.58 ± 0.47	1.25 ± 0.12	0.65 ± 0.14	13.14 ± 12.77
0.01	1.24 ± 0.05	0.54 ± 0.04	4.98 ± 0.47	1.33 ± 0.11	0.52 ± 0.08	1.51 ± 1.37
0.1	1.29 ± 0.25	0.49 ± 0.09	6.96 ± 1.86	1.55 ± 0.09	1.02 ± 0.73	1.91 ± 1.80
1	1.52 ± 0.06	0.52 ± 0.03	4.87 ± 0.19	1.55 ± 0.06	0.39 ± 0.03	0.84 ± 0.06

Dose (% in feed)	Relative Organ Weight (g/g brain weight)				
	Heart	Liver	Kidney	Spleen	Ovary
0	0.33 ± 0.01	3.25 ± 0.45	1.14 ± 0.12	0.59 ± 0.12	11.80 ± 11.46
0.01	0.44 ± 0.05	4.02 ± 0.38	1.08 ± 0.10	0.42 ± 0.05	1.31 ± 1.20
0.1	0.38 ± 0.01	5.89 ± 2.56	1.26 ± 0.31	0.93 ± 0.75	1.81 ± 1.24
1	0.34 ± 0.04	3.21 ± 0.01	1.02 ± 0.09	0.26 ± 0.03	0.56 ± 0.06

a/ Mean ± standard error of two mice.
b/ Mean ± standard error of three mice.

TABLE 98

SUMMARY OF TISSUE LESIONS IN MALE MICE FED TNG FOR 24 MONTHS

Dose (% in feed):	0	0.01				0.1	
Mouse No.:	131	342	349	355	358	521	551
Treatment-Related Lesions[a]							
Liver							
Pigmented macrophages						1	2
Other Lesions							
Eye			0		0		
Retinal degeneration	X			X		X	
Trachea							
Retained inspissated secretion				X			
Lung							
Chronic pneumonia		+	2	2			
Bronchoalveolar adenoma							X
Heart							
Amyloid deposits	2		1	2			2
Focal myocarditis		±					
Mural thrombosis of atrium			X		X		
Myocardial scarring			1	2	2	2	
Hemangioendothelioma							X
Stomach							
Amyloid deposits			X				
Liver							
Dysplasia, minimal	X		X	X	X	X	X
Amyloid deposits			1	2	1		
Multiple granulomata		3					
Chronic pericholecystitis						X	
Hemangioendothelioma							X
Intestine							
Amyloid deposits	X	X	X	X	X	X	X
Pinworm	X	X		X			X
Kidney							
Amyloid deposits	1	3	4	3	3	3	3
Retention cysts	±						
Testis							
Focal atrophy	1	1	3	3	2		
Hypospermia	1	1	3		2		
Interstitial cell hyperplasia		3		2			
Seminal Vesicle							
Degeneration			1		3		
Generalized Amyloidosis		X	X	X	X		
Adrenal Gland							
Aging changes	1	2	3	2	3		
Amyloid deposits		1	2		3		
Cortical atrophy		2	3	2	3	2	1
Thyroid Gland			0				
Aging changes	1						
Amyloid deposits				3	3		
Colloid cysts		2				1	2
Medullary degeneration					3		
Atrophy of follicular epithelium				3	3	2	
Spleen							
Splenomegaly			X	X			
Amyloid deposits			2	3	1		
Hemangioendothelioma		X					X
Lymph Node				0			
Amyloid deposits	1	1					
Hemangioendothelioma							X
Bone Marrow							
Hypoplasia		2					

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 89

SUMMARY OF TISSUE LESIONS IN FEMALE CONTROL MICE FOR MICE FED TNG FOR 24 MONTHS

Mouse No.:	219	224	227	232	241	243	244	247	248	249	250	252	254	255
Lesions[a/]														
Eye	0	0												
Retinal degeneration				X	X	X		X	X	X	X	X	X	X
Trachea														
Chronic tracheitis								1						
Retained inspissated secretion		X	X	X		X								
Lung														
Chronic pneumonia											2			
Broncho-alveolar adenoma					X									
Heart														
Amyloid deposits			2	1						±		±		
Myocardial scarring													1	1
Artery (renal)														
Intimal thickening												1		
Salivary Gland														
Focal siladenitis				1										
Amyloid deposits			1									1		
Stomach														
Acute ulcer					X									
Glandular hyperplasia													2	
Liver														
Dysplasia, minimal	X			X	X		X	X		X	X		X	
Cholecystitis				X										
Hemangioma	X													
Intestine														
Amyloid deposits	1		3	3								1	1	
Pinworms			X											X
Kidney														
Amyloid deposits	3	3	3	3						1		3		
Chronic interstitial nephritis	1	1					±	1			1			±
Tubular degeneration		2												
Glomerular sclerosis					2									
Renal cell hyperplasia											1			
Ovary														
Hyalinization			3											
Stromal hyperplasia											1			
Pigment deposits										2				
Cystadenoma	X				X									
Uterus		0												
Endometrial hyperplasia	1		1	1		1	1	1	1				3	3
Abcessation			X											
Adrenal Gland		0												
Aging changes	3		1	2	1	1	2	1	1	3	2	2	2	3
Amyloid deposits												3		
Pigment deposits	2		1		1		1							
Thyroid Gland							0	0						
Aging changes	±	1	±		±	±				±	±		±	2
Follicular carcinoma				X										
Spleen														
Amyloid deposits												1		
Pigment deposits			2											
Malignant lymphoma									X					
Lymph Node		0				0								
Malignant lymphoma									X					

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 90

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 0.01% TNG FOR 24 MONTHS

Mouse No.:	420	427	428	432	433	440	445	447	448	451	453	455
Lesions[a/]												
Eye												
Retinal degeneration	X		X		X	X	X	X		X	X	X
Skin												
Lymphocytic infiltration	X											
Malignant lymphoma				X								
Trachea												
Retained inspissated secretions		X			X							X
Lung												
Chronic pneumonia			1	2	2	2				2		3
Bronchoalveolar adenoma					X		X		X	X		
Heart												
Amyloid deposits	2	2	2								1	+
Parotid Gland												
Focal hyalinization											X	
Liver												
Dysplasia, minimal					X	X		X	X	X	X	
Amyloid deposits					X							
Fatty change								X				
Centrolobular necrosis							3					
Granulomatous hepatitis	1	2	1	2				+	1	1	+	2
Hemangioma							X					
Intestine												
Amyloid deposits	X	X	X		X		X		X		X	X
Pinworm		X			X		X	X		X		X
Kidney												
Amyloid deposits	2	3	4		4		3	2	3		3	
Chronic interstitial nephritis						3	1					1
Tubular degeneration						2			2	3		
Tubular epithelial degeneration												2
Glomerulosclerosis				X								
Ovary												
Amyloid deposits		X									X	X
Benign cyst					X							
Stroma lutenization								X		X		
Follicular cell tumor			X						X			
Serous cystadenoma						X						
Hemorrhagic benign cyst							X		X			
Uterus												
Cystic change	X	X	X		X		X			X	X	X
Hemangioma				X								
Generalized Amyloidosis	X	X	X		X		X		X		X	
Brain												
Focal cortical necrosis					X							
Adrenal Gland												
Aging changes	1	2	2	1		2	1			2		2
Amyloid deposits		2	2		2		2				2	
Cortical atrophy	1	2			3	2				2	2	2
Paraganglioma with extensions								X				
Pituitary Gland												
Developmental stalk cyst												X
Basophilic hyperplasia					2							
Chromophobe adenoma				X		X	X				X	

TABLE 90 (concluded)

Mouse No.:	420	427	428	432	433	440	445	447	448	451	453	455
Thyroid Gland												
Atrophy of follicular epithelium	2	1	2									3
Colloid cysts	2	2		2	3			1				2
Amyloid deposits			1									
Spleen												
Extramedullary hematopoiesis			X									
Malignant lymphoma				X								
Lymph Node												
Amyloid deposits							1					
Lymphoid hyperplasia	X		X			X	X	X	X		X	X
Mastocytosis					X							
Plasmacytosis		X										
Malignant lymphoma				X								
Bone Marrow												
Hypoplasia		2	2		2	2	2	2	2	2	3	2
Depressed erythropoiesis		2	2					3			3	2
Depressed maturation of granulocytes					2	2	3		2	3		

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 91

SUMMARY OF TISSUE LESIONS OF FEMALE MICE FED 0.1% OR 1% TNG FOR 24 MONTHS

Dose (% of feed)	0.1								1					
Mouse No.:	621	623	630	640	643	645	655	656	841	843	848	850	851	855
Treatment-Related Lesions[a]														
Liver														
Pigment deposits	1	±				±		1		1	1	±	1	±
Ovary														
Pigmented stromal cells				2			3				1			
Adrenal Gland														
Pigment deposits												±		
Spleen												0		
Excessive pigment											1		1	
Other Lesions														
Eye														
Retinal degeneration					X	X	X	X		X				
Skin														
Subcutaneous abcess									X					
Trachea														
Retained inspissated secretions								X		X			X	
Lung														
Chronic pneumonia				3							2			
Lymphocyte aggregates							2							
Pleural thickening														1
Bronchoalveolar adenoma			X							X			X	
Heart														
Amyloid deposits	2					±						±		
Focal myocarditis							±							
Myocardial scarring										1				
Salivary Gland														
Periparotid abcess													X	
Stomach														
Amyloid deposits	1													
Metaplasia and chronic inflammation of glandular stomach														1
Liver														
Dysplasia, minimal	X		X	X	X	X	X		X		X	X	X	X
Amyloid deposits	2	1												
Granulomata									±	1				
Degeneration with regeneration		1						2						
Intestine														
Amyloid deposits	X	X		X		X					2	1		
Pinworms						X					1			1
Kidney														
Amyloid deposits	3	3	2	3		3		4			2	3		
Chronic interstitial nephritis				2	1	1	2	1		1	2	1	1	
Retention cysts					X									
Urinary Bladder														
Cystitis follicularis											X			

TABLE 91 (concluded)

Dose (% of feed)	0.1								1					
Mouse No.:	621	623	630	640	643	645	655	656	841	843	848	850	851	855
Other Lesions (concluded)														
Ovary														
Amyloid deposits	3													
Hemorrhagic bursa ovarica		X												
Serous cystadenoma	X						X							
Pseudomucinous cystadenoma											X			
Follicular cell tumor						X				X			X	
Uterus														
Cystic endometrial hyperplasia		2	3	1	1	3	1	1			1		1	
Adrenal Gland							0							
Aging changes		3	2	1	1	3			1	2	1	1	±	1
Amyloid deposits	3	3						3			1	2		
Cortical atrophy	3													
Cortical adenoma					X									
Thyroid Gland					0		0							
Amyloid deposits	3					2		2						
Atrophy of follicular epithelium	3	1		2		3		3				1		
Colloid cysts			2	1										
Spleen														
Splenomegaly	X	X						X						
Amyloid in red pulp								X						
Bone Marrow														
Left shift of cells						1		1						
Hypoplasia							X				1		1	1

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 92

SUMMARY OF TISSUE LESIONS OF MALE CONTROL MICE DYING AT UNSCHEDULED TIMES

Mouse No.:	144	106	145	115	116	130	143	123	136	158	112	119	154	155	138
Week of Death:	9	48	49	57	62	62	73	77	77	77	82	82	82	82	93
Lesions[a/]															
Eye			0	0	0				0						
Retinal degeneration	X	X				X	X	X		X	X	X	X		X
Skin									0						
Subcutaneous multilocular cysts				X											
Subcutaneous spindle cell sarcoma					X										
Trachea		0	0	0											
Retained inspissated secretion	1						2								
Lung															
Chronic pneumonia			2					1						1	
Malignant reticulosis inpericardium									X						
Heart															
Amyloid deposits	2	1	1	1	2		1			1		1	±		
Mural thrombus					3						2	2			
Myocardial scarring							1	1			1			1	1
Malignant reticulosis									X						
Salivary Gland															
Amyloid deposits			1												
Regressive-degenerative change	1														
Liver															
Dysplasia, minimal	X		X	X	X	X	X	X		X	X	X	X		X
Amyloid deposits			1		1		±								
Degeneration														1	
Malignant reticulosis									X						
Pancreas															
Lymphocytic infiltrate	1														
Intestine															
Amyloid deposits	1		2	1	2		2				2		2	1	2
Pinworm	X						X				X				X
Kidney															
Amyloid deposits	3		4	2	4		2	2		2	2	2	2	3	2
Chronic interstitial nephrotis						1							2	2	
Malignant reticulosis (including perirenal fat)									X						
Testis															
Focal atrophy	2	1	1					1		3					
Hypospermia	3														
Adrenal	0	0	0	0		0			0	0				0	
Amyloid deposits					3		3	3				3	2		1
Aging changes					3		3	3			3	3	2		1
Thyroid		0		0		0			0						
Amyloid deposits							3								
Aging changes	±		±		±			2		±	1	±	±	±	±
Spleen															
Malignant reticulosis									X						
Lymph Node															
Reactive lymphoid hyperplasia													2		
Malignant reticulosis									X						

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 93

SUMMARY OF TISSUE LESIONS OF MALE MICE FED 0.01% TNG AND DYING AT UNSCHEDULED TIMES

Mouse No.:	324	351	314	323	309	321	347	312	317	345	337	311	340	343	357	339	356	326	334	353
Week of Death:	12	29	58	62	62	66	66	66	68	69	72	78	78	78	78	84	84	90	93	99
Treatment-Related Lesions[a]																				
Kidney																				
Pigment deposit				1												±				
Other Lesions																				
Eye	0	0		0			0				0									
Retinal degeneration					X	X		X	X			X		X	X	X		X	X	X
Harderian Gland																				
Lymphangioma														X						
Trachea											0		0							
Retained inspissated secretions										X									X	
Lung																				
Chronic pneumonia			2	2			1				3				2			2		
Early acute pneumonia																			X	
Intra-alveolar and interstitial hemorrhage, massive	X																			
Bronchoalveolar tumor															X					
Myelocytic leukemia		X																		
Heart																				
Amyloid deposits														2		3	3	1	1	1
Mural thrombus			X	X					X					X	X	X	X		X	X
Myocardial scarring			1	2	1	2	2	1	2	1	2	1	2	2	2	2				
Interstitial edema																				1
Intravascular thrombus													X							
Myelocytic leukemia		X																		
Stomach																				
Amyloid deposits				X	X								X		X	X			X	
Acute ulcer																				X
Liver																				
Dysplasia, minimal	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Amyloid deposits																2				
Passive hyperemia	3																			
Fatty change				1																
Centrilobular necrosis																	2			
Myelocytic leukemia		X																		
Pancreas																				
Amyloid deposits				1									1							
Duct cyst													X							
Intestine									0		0									
Amyloid deposits			1	1	3	2	2	1		1		1		1	1	1	2	2	2	1
Pinworm			X									X				X			X	
Kidney																				
Amyloid deposits				3	4	4	4	4	3	3	3	4	3	3	3	4	3	3	4	3
Chronic interstitial nephrotis										1	1	1	2		2	1	1			
Arteriosclerosis of renal artery	3					3														
Passive hyperemia																				
Cortical retention cysts											X					X	X		X	
Focal calcification																	1			
Focal granuloma in hilus																			X	
Myelocytic leukemia	X																			
Urinary Bladder																				
Focal ulcerative cystitis																	X			
Testis																				
Focal atrophy			1	3	2	3	3		1		±		1	2		2		2	2	
Hypospermia				3	3	4	3		2					3	2	2	2		3	2
Vascular changes (intimal proliferation)							2													
Amyloid deposits														2						
Myelocytic leukemia	X																			

TABLE 93 (concluded)

Mouse No.:	324	351	314	323	309	321	347	312	317	345	337	311	340	343	357	339	356	326	334	353
Week of Death:	17	29	58	62	62	66	66	66	68	69	72	78	78	78	78	84	88	90	93	99
Other Lesions (concluded)																				
Epididymis	0																			
Epithelial degeneration								1												
Seminal Vesicle																				
Degenerative changes				2	2	2		1	2	2	1	2	1							1
Generalized Amyloidosis				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Skeletal Muscle																				
Granular myoblastoma															±					
Adrenal Gland	0	0		0	0			0	0	0	0								0	
Amyloid deposits							2					2	2	3	2	3	1	2		2
Aging changes			2				2					2	2		2	3	1			2
Cortical atrophy						2	2					2	2	3	2			2		1
Pituitary	0	0	0	0		0	0	0	0	0	0					0				
Adenoma																	X			
Thyroid	0	0	0		0		0	0			0							0	0	
Amyloid deposits				2									2	3	1	2				
Atrophy of follicular epithelium				3		2			3	2		3		3	1	3	2			2
Colloid cyst										2		2	2		2		2			2
Follicular adenoma									X											
Spleen																				
Splenomegaly		X										X								
Amorphia from leukemic blast cells		X																		
Amyloid deposits			2	1	3		?	1	1	3				3		3				
Extramedullary hematopoiesis												3								
Depletion of cellular elements																3	2		2	
Lymph Node																				
Amyloid deposits												X			X			X		
Plasma cell hyperplasia												3								
Sinusoidal lining cell hyperplasia												2								
Lymph cyst												X								
Bone Marrow	0	0			0		0				0									
Hypoplasia			3	2		±		2	±	2		?	±							

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 94

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 0.1% OR 1% TNT AND DYING AT UNSCHEDULED TIMES

Dose (% in feed):	0.1									1						
Mouse No.:	520	501	536	517	546	524	516	516	515	721	740	747	733	750	751	754
Week of Death:	26	53	63	65	78	86	90	92	101	38	52	62	68	70	74	96
Treatment-Related Lesions[a/]																
Liver																
Pigment deposits									±	1	2	±	1	±	±	±
Kidney																
Pigment deposits															±	
Spleen																
Excessive pigment												±	1			
Other Lesions																
Eye		0	0	0			0	0								0
Retinal degeneration	X				X	X				X	X			X		
Trachea																
Retained inspissated secretions	2															
Lung																
Chronic pneumonia			2								2					
Heart																
Amyloid deposits	1			1	1				2		1	2	3	1	2	
Mural thrombus					3		3	3								
Myocardial infarct							2									
Myocardial scarring			1		1	1	2	1								
Chronic myocarditis																2
Stomach																
Epithelial metaplasia													2	1		
Ulcer													X			
Liver																
Dysplasia, minimal	X	X	X	X	X	X	X	X	X		X			X	X	
Amyloid deposits		1	1		±			1				1	2		2	
Passive hyperemia							3									
Hepatocyte degeneration										1		1	2			1
Pancreas																
Cystoma						X										
Intestine																
Amyloid deposits		X	X		X	X			X			2	2	2		2
Pinworm		X							X		X					
Kidney																
Amyloid deposits		3	3	3	3	2	3	3	3		3	3	3	3	3	
Chronic interstitial nephritis	4										1	±	1		2	
Glomerulosclerosis						2										
Tubular degeneration															1	
Solid tubular renal adenoma									X							
Urinary Bladder																
Chronic cystitis with ulceration and squamous metaplasia	4	1														
Testis							0									0
Atrophy		2	3					3				1	2		2	
Focal calcification		±	3								1		2	1		
Skin																
Ulcer with inflammation									X							
Subcutaneous hemangioma							X									
Abdominal Cavity																
Sarcoma										X						
Adrenal Gland				0			0									
Amyloid deposits		2	3			2						3	3	3	3	3
Aging changes											1					
Cortical atrophy		3	3		3	3			3	2						
Calcification of zona intermedia									2							
Fibrosis of medulla											2					
Pituitary Gland																
Cyst								X								
Thyroid Gland			0	0		0	0		0				0			0
Amyloid deposits		1										3		2	3	
Atrophy of follicular epithelium	2	3			2											
Colloid cysts		2			2					2						
Spleen								0								0
Amyloid deposits		2			4							3		1	2	
Splenomegaly					X				X							
Hyperplasia of megakaryocytes										1						
Lymphoid atrophy												?	!		2	
Lymph Node											0		0!			0
Lymphocyte depletion														1	2	
Bone Marrow													0!			
Hypoplasia			±			+		±		±	1	3		±	2	

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 95

SUMMARY OF TISSUE LESIONS IN FEMALE CONTROL MICE DYING AT UNSCHEDULED TIMES

Mouse No.:	257	233	214	235	226	221	258	259	220	222	223	240	213
Week of Death:	30	48	50	62	84	84	84	91	92	98	99	100	105[b]
Treatment-Related Lesions[a]													
Lymph Node													
Pigment deposits										1			
Other Lesions													
Eye	0	0	0							0			
Retinal degeneration					X				X		X	X	
Trachea	0		0	0						0			
Retained inspissated secretions		1				1						1	
Lung													
Chronic pneumonia				3			1						
Bronchoalveolar adenoma													X
Malignant lymphoreticulosis			X			X							
Heart													
Amyloid deposits							1				1		
Chronic myocarditis		±								±			
Malignant lymphoma			X										
Salivary Gland													
Amyloid deposits									1				
Sialadenitis							1						1
Liver													
Dysplasia, minimal	±	X		±	±		X		X	X	X	X	X
Hemangioendothelioma											X		
Malignant lymphoreticulosis						X							
Intestine	0	0	0							0			
Amyloid deposits					1		2				1		1
Pinworm				X	X	X	X		X		X		
Malignant lymphoma									X				
Kidney													
Amyloid deposits							3				3		?
Chronic interstitial naphritis	3	1		3						1			
Glomerulosclerosis	3	3		3									
Tubular degeneration	2	2					±						
Malignant lymphoma			X			X							
Ovary													
Hemorrhage												X	
Stromal hyperplasia		1					3						
Malignant lymphoma						X			X				
Uterus													
Cystic endometrial hyperplasia	1						3						
Leiomyosarcoma								X					
Malignant lymphoma			X										
Skin													
Subcutaneous hemangioma							X						
Subcutaneous fibrosarcoma											X		
Brain													
Malignant lymphoma in pia-arachnoid									X				
Adrenal Gland	0	0	0	0						0		0	
Amyloid deposits							2				2		2
Aging changes					2	3		2	±		2		1
Thyroid Gland	0	0	0	0						0			
Atrophy							2				3		2
Colloid cysts					2	±		±				±	
Spleen													
Amyloid deposits							1						
Splenomegaly (due to lymphoma)			X			X			X				
Lymph Node													
Hyperplasia								1					
Malignant lymphoma			X			X			X				
Bone Marrow	0	0	0	0					0				0
Hyperplasia						1							

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

b/ Died in first week of recovery after 24 months of feeding TNG.

TABLE 96

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 0.01% TNG AND DYING AT UNSCHEDULED TIMES

Mouse No.:	409	452	418	429	422	414	434	424	421	436	449	442	443	459
Week of Death:	37	38	44	78	80	90	92	95	96	97	99	100	100	104
Treatment-Related Lesions[a/]														
Kidney														
Pigment deposits							3							
Other Lesions														
Eye	0	0	0										0	0
Retinal degeneration							X				X	X		
Lung														
Chronic pneumonia	2						2			2				
Passive hyperemia			3										3	
Malignant reticulosis	X	X												
Malignant lymphoma						X								
Heart														
Amyloid deposits										±				
Mural thrombus													3	
Myocardial scarring														2
Pericarditis			1						1					
Malignant reticulosis	X	X												
Aorta														
Periaortic granulation tissue			X											
Salivary Gland														
Amyloid deposits										1				
Malignant lymphoma						X								
Liver														
Dysplasia, minimal				X	X			X						
Fatty change						?								
Focal necrosis							1		2				3	
Passive hyperemia			3											
Granulomatous hepatitis										3				
Cell unrest													3	
Centrolobular necrosis														3
Malignant reticulosis	X	X												
Malignant lymphoma											X			
Pancreas														
Malignant lymphoma						X								
Intestine														
Amyloid deposits					X			X		X			X	
Pinworms									X	X	X	X		
Kidney														
Amyloid deposits													3	
Chronic interstitial nephritis					1				1					1
Glomerulosclerosis			1					2						
Tubular degeneration							3							
Malignant reticulosis	X	X												
Malignant lymphoma											X			
Urinary Bladder														
Focal cystitis										X				
Cystitis follicularis							X							

TABLE 96 (concluded)

Mouse No.:	409	452	418	429	422	414	434	424	421	436	449	442	443	459
Week of Death:	37	38	44	78	80	90	92	95	96	97	99	100	100	104
Other Lesions (concluded)														
Ovary			0											
Benign cysts							X							
Follicular cell tumor	X													
Papillary cystadenoma										X	X			X
Bursa Ovarii														
Malignant reticulosis	X	X												
Uterus														
Acute purulent endometritis							3							
Cystic dilatation of endometrial glands										X	X	X		X
Malignant lymphoma						X								
Adrenal Gland	0	0	0			0		0					0	
Atrophy				2										
Aging changes					2				2	2	3	2		2
Thyroid Gland	0	0		0			0	0	0	0		0	0	
Atrophy of follicular epithelium			2			2								
Colloid cysts					2									2
Malignant lymphoma						X								
Spleen														
Passive hyperemia			3											
Lymphocyte depletion			2											
Extramedullary hematopoiesis								X						
Splenomegaly with focal granulomas										X				
Calcium-iron incrustations			X											
Malignant reticulosis	X	X												
Malignant lymphoma						X					X			
Lymph Node														
Benign hyperplasia									1	2				1
Histiocytic lymphoma						X					X			
Malignant reticulosis	X	X												
Bone Marrow	0	0	0										0	
Hypoplasia				2	2	2	2	2	2	2	2	2		2
Erythroid depression				3				2		3		2		

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 97

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 0.1% OR 1% TNG AND DYING AT UNSCHEDULED TIMES

Dose (% in feed):	0.1												1								
Mouse No.:	612	606	646	632	617	618	611	616	631	633	610	629	652	848	845	825	844	819	822	823	852	837
Week of Death:	25	52	60	65	68	86	94	94	96	96	97	100	104	36	64	70	78	91	92	92	97	106[b]
Treatment-Related Lesions																						
Liver																						
Pigment deposits							1			±					2	±	±			±	±	
Kidney																						
Pigment deposits										1						2						
Thyroid Gland																						
Pigment deposits															±							
Spleen																						
Excessive pigmentation															2			1			1	
Other Lesions																						
Eye	0				0			0					0	0	0			0				
Retinal degeneration				X							X								X	X		
Trachea																						
Retained inspissated secretions	X				X				X											X		
Lung																						
Chronic pneumonia							2	2				2						1	1			
Peribronchial lymphoid aggregates													1									
Bronchoalveolar adenoma			X						X	X		X									X	X
Collision tumor (squamous cell carcinoma invading bronchoalveolar tumor)			X																			
Heart																						
Amyloid deposits					1		1									1	±	1	1	±	±	
Chronic myocarditis						±						±									±	
Atherosclerosis																1						
Liver																						
Dysplasia, minimal	X		X		X	X				X	X		X			X	X	X	X	X		
Amyloid deposits					1	1										2		2	1	2	1	
Steatosis	2		1						2													
Degeneration and regeneration							1					1										
Degeneration																					2	
Focal calcification																X						
Malignant leukemia		X																				
Malignant reticulosis								X														
Histiocytic lymphoma				X																		
Intestine																		0				
Amyloid deposits					2	2	3				1					2	2		2	1	2	
Pinworms								X	X			X				X	X		X			X
Kidney																						
Amyloid deposits					4	3	3				1	3				3	3	3	3	3	3	
Chronic interstitial nephritis	±					2	2		2						1							
Focal calcification																1		±				
Malignant leukemia		X																				
Histiocytic lymphoma				X																		
Ovary								0	0													
Amyloid deposits					3		2												2			
Hemorrhage in Bursa ovarica										X												
Stromal hyperplasia																	1					
Follicular cell tumor							X															
Hemangioma						X																
Serous cystadenoma											X	X										
Malignant leukemia		X																				
Lipoid cell tumor																					3	
Uterus																0		0				
Cystic hyperplasia					1	3	2		1	1												
Endometrial hyperplasia															1		1			1	1	3
Purulent endometritis																			3			
Hemangioma																			X			
Malignant reticulosis								X														

TABLE 97 (concluded)

Dose (% in feed):	0.1													1						
Mouse No.:	612	606	646	632	617	618	611	616	631	633	610	629	652	848	845	825	844	819	822	823	852	837
Week of Death:	25	52	60	65	68	86	94	94	96	96	97	100	104	36	64	70	78	91	92	92	97	106[b]
Other Lesions (concluded)																						
Mammary Gland																						
Carcinoma		X																				
Benign tumor, ulcerated																					X	
Skin																						
Ulcer													X									
Subcutaneous cyst								X														
Cystically dilated sweat glands																			X			
Subcutaneous fibrosarcoma							X					X										
Squamous cell carcinoma																						X
Bone																						
Osteosarcoma														X								
Brain																						
Malignant leukemia		X																				
Adrenal Gland	0		0	0												0	0					
Amyloid deposits					3	3	2					2	2					3	3	3	3	
Cortical atrophy					2	3	1															
Aging changes						3	1		1	2	1	2	2		2			2			2	2
Abcess at cortico-medullary junction							X															
Malignant leukemia		X																				
Thyroid Gland				0								0		0	0	0	0	0				
Amyloid deposits					3	3	2												3	3	3	
Aging changes																						±
Atrophy of follicular epithelium					3	3	2			1	2											
Colloid cysts					2				±	2												
Adenomatous goiter			X																			
Spleen																						
Amyloid deposits					2																	
Reactive hyperplasia						2	1		1	1												
Cyst												X										
Histiocytic lymphoma				X																		
Malignant leukemia		X																				
Lymph Node																						
Reactive hyperplasia						2	1		1	1												
Lymphocyte depletion					2																	
Lymphoid hyperplasia																	2					
Histiocytic lymphoma				X																		
Malignant leukemia		X																				
Squamous cell carcinoma			X																			
Malignant reticulosis								X														
Bone Marrow	0			0										0				0				
Hypoplasia			±		±	±	±	±	±		±				1				1		1	1
Granulocytic leukemia		X																				

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not be read.

b/ Died in 2nd week of recovery after 24 months of feeding TNG.

TABLE 98

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED TNG FOR 24 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0		0.01			0.1		1	
Mouse No.:	211	216	413	416	419	609	615	810	812
Treatment-Related Lesions[a]									
Liver									
Pigment deposits								+	1
Ovary									
Pigment deposits								2	
Other Lesions									
Eye				0					
Retinal degeneration	X	X	X		X				
Trachea									
Retained inspissated secretions			X	X	X			X	X
Lung									
Chronic pneumonia			2						1
Bronchoalveolar adenoma		X	X		X		X		
Malignant reticulosis						X			
Heart									
Focal myocarditis									+
Arteries									
Focal panarteritis			X	X					
Stomach									
Glandular metaplasia				X					
Liver									
Dysplasia, minimal		X	X	X	X		X	X	X
Granulomatous hepatitis			1		+				
Malignant reticulosis						X			
Intestine									
Amyloid deposits						X		X	
Pinworms		X	X					X	
Kidney									
Amyloid deposits								3	
Chronic interstitial nephritis	1	1	1	1	1		1	1	
Glomerulosclerosis				2	1				
Cortical retention cysts							X		
Malignant reticulosis						X			
Ovary									
Stromal hyperplasia		1						2	
Stromal luteinization			X		X				
Mast cell infiltration					X				
Hemorrhagic bursa ovarica	4								
Cystic change			X	X					
Serous cystadenoma				X					
Hemangioma								X	
Uterus									
Endometrial hyperplasia	1	3						2	
Cystic endometrial hyperplasia			X	X			X		
Hemangioendotheliona						X			

TABLE 98 (concluded)

Dose (% in feed):	0		0.01			0.1		1	
Mouse No.:	211	216	413	416	419	609	615	810	812
Other Lesions (concluded)									
Brain									
Calcification	X								
Adrenal Gland									
Amyloid deposits								2	
Aging changes	3		2		2	2	2	2	1
Pituitary Gland									
Developmental stalk cyst					X				
Basophilic hyperplasia			2						
Chromophobe adenoma				X					
Thyroid Gland	0								
Amyloid deposits						1		1	
Aging changes		±							
Atrophy of follicular epithelium			2	1	2	1	1		
Colloid cysts			1	1	1				
Spleen									
Malignant reticulosis						X			
Lymph Node									
Lymphoid hyperplasia	2		X		X				
Malignant reticulosis						X			
Bone Marrow									
Hypoplasia			3	2	2			2	2
Depressed erythropoiesis			3	3	2				
Malignant reticulosis						X			

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild, 2 = moderate, 3 = severe, 4 = very severe, ± = minimal or questionable, X = present, 0 = tissue missing or could not read.

TABLE 99

TUMOR OCCURRENCE IN MICE FED TNG

Dose (% in feed):	0		0.01		0.1		1	
Sex:	Male	Female	Male	Female	Male	Female	Male	Female
Sites, Tumors								
Harderian Gland								
Lymphangioma			1[a/]					
Lung								
Bronchoalveolar adenoma		3	1	6	1	6		4
Squamous cell carcinoma						1		
Heart								
Hemangioendothelioma					1			
Liver								
Hemangioma		1		1				
Hemangioendothelioma					1			
Pancreas								
Cystoma					1			
Ovary								
Cystadenomas		1		2		4		1
Follicular cell tumor				2		1		2
Lipoid cell tumor								1
Hemangioma						1		1
Uterus								
Hemangioma								1
Hemangioendothelioma						1		
Leiomyosarcoma		1						
Mammary Gland								
Carcinoma						1		
Kidney								
Tubular adenoma					1			
Skin and Subcutaneous Tissue								
Sarcomas	1						1	
Fibrosarcoma						2		
Squamous cell carcinoma								1
Hemangioma					1			
Bone								
Osteosarcoma								1
Skeletal Muscle								
Myoblastoma			1					
Multiple Sites								
Malignant leukemia			1			1		
Malignant lymphoma		2		2				
Malignant reticulosis	1			2		2		
Malignant lymphoreticulosis		2						
Histiocytic lymphoma		2		1				

TABLE 99 (Concluded)

Dose (% in feed):	0		0.01		0.1		1	
Sex:	Male	Female	Male	Female	Male	Female	Male	Female
Sites, Tumors								
Adrenal Gland								
Paraganglioma				1				
Cortical adenoma						1		
Pituitary Gland								
Chromophobe adenoma			1	5				
Spleen								
Hemangioendothelioma					1			
Lymph Node								
Hemangioendothelioma					1			

a/ Number of mice with the lesion.

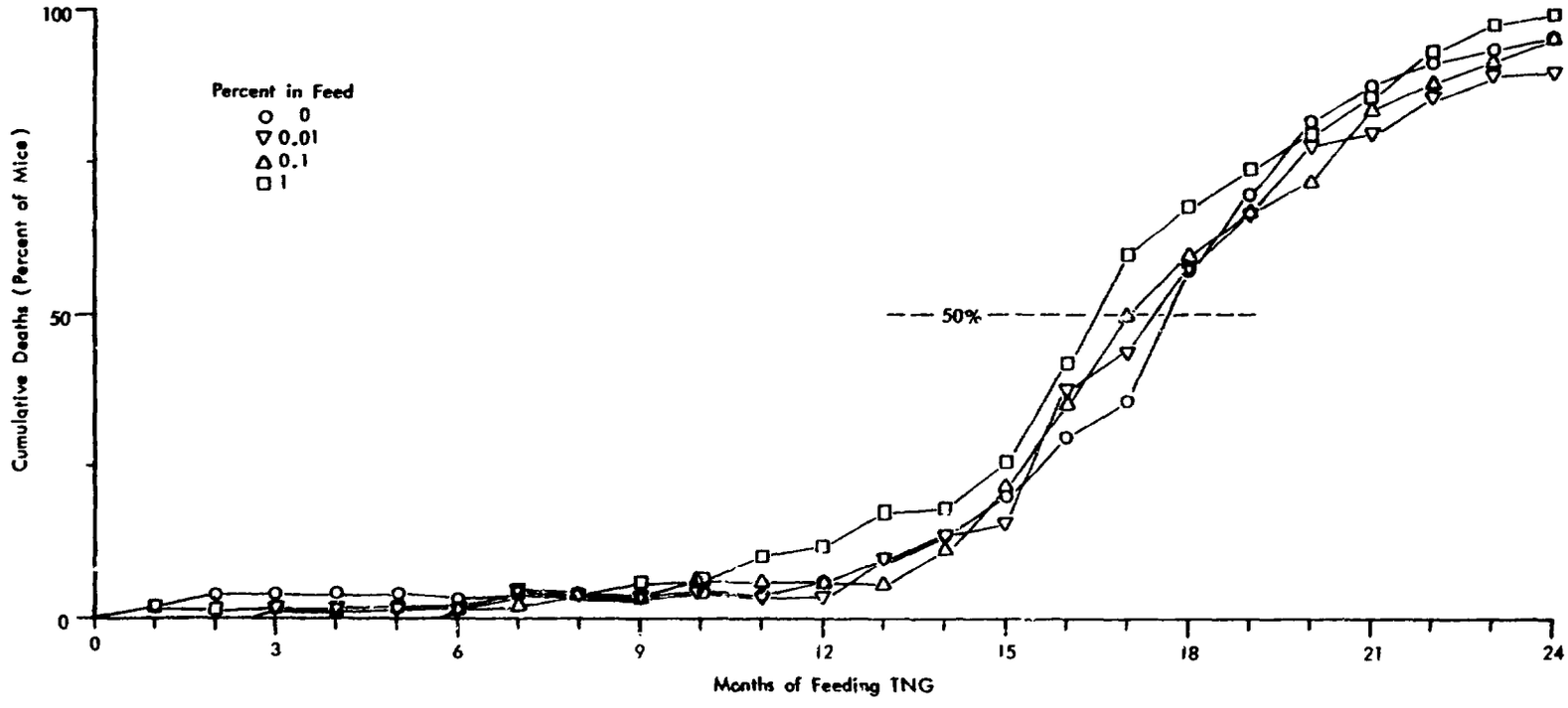

Figure 16 - Cumulative Unscheduled Deaths Among Male Mice Fed TNG

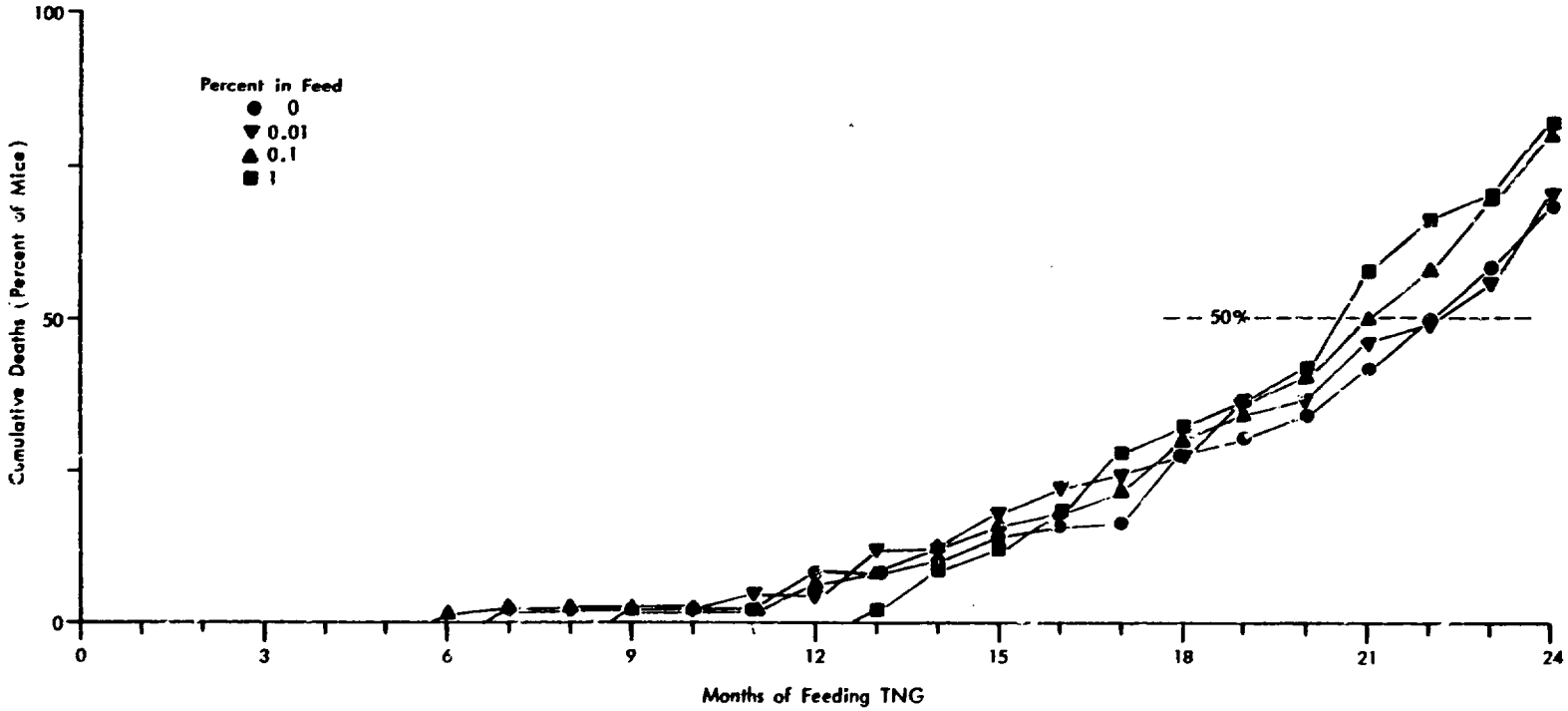

Figure 17 -- Cumulative Unscheduled Deaths Among Female Mice Fed TNG

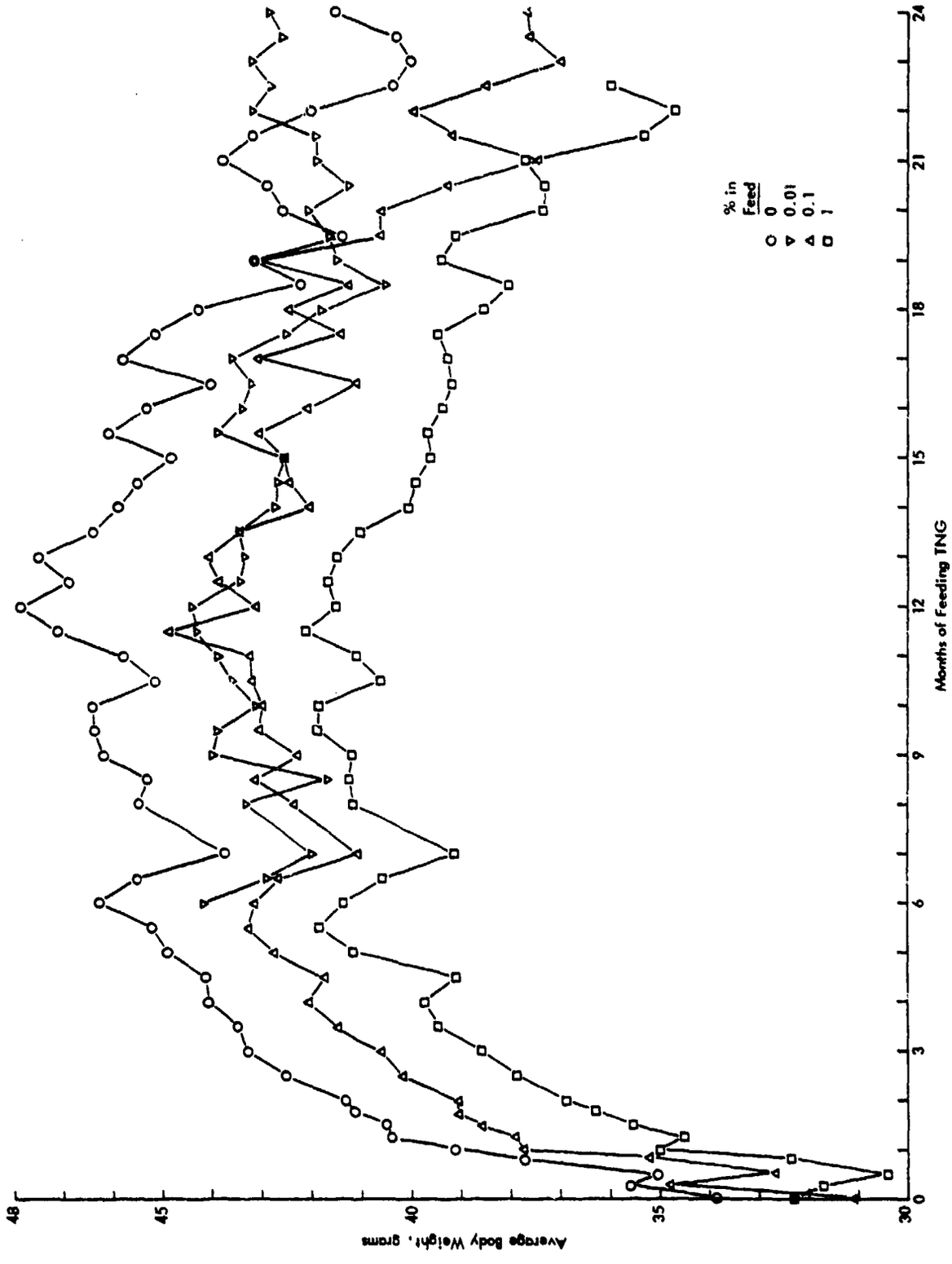

Figure 18 - Average Body Weights of Male Mice Fed TNG

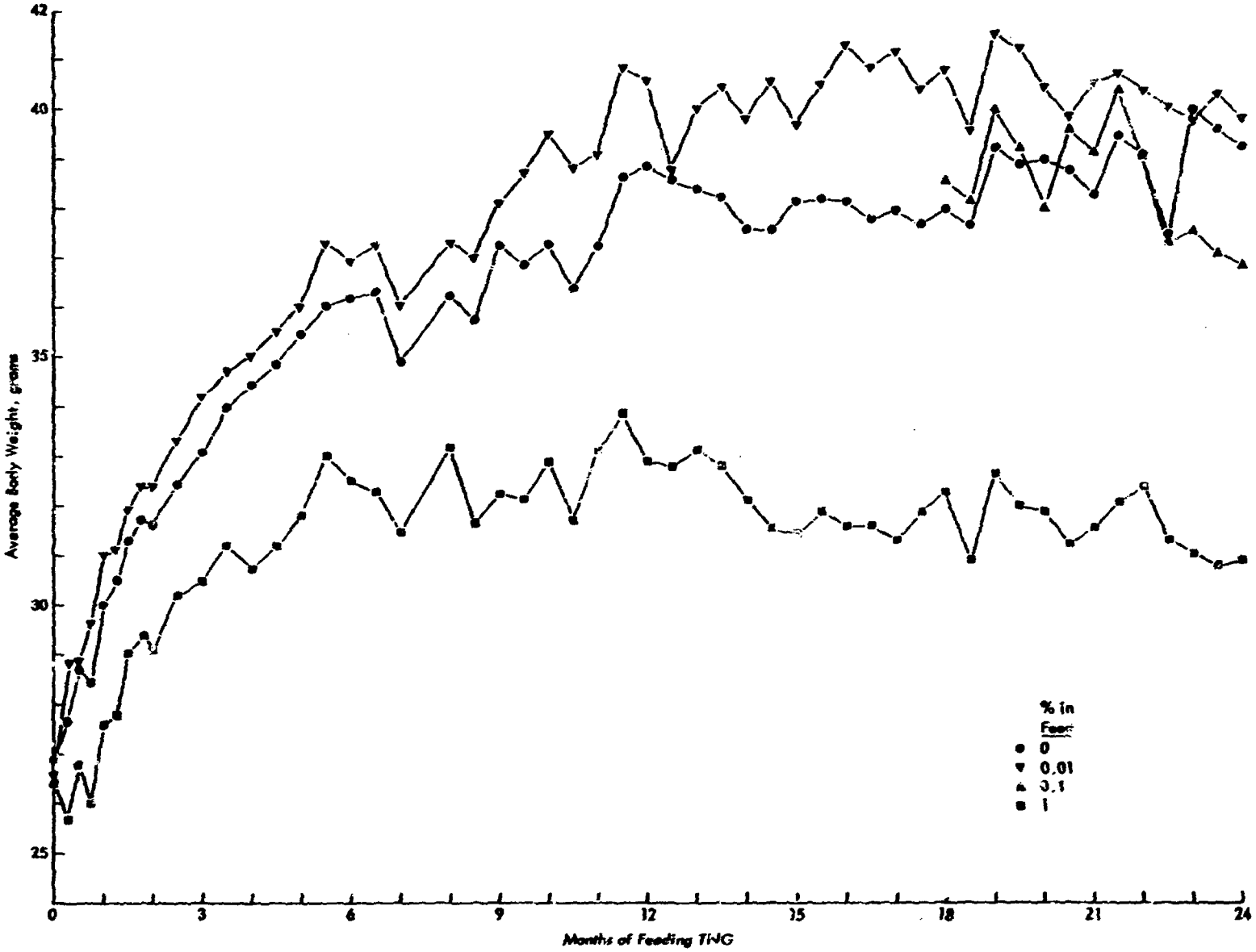

Figure 19 - Average Body Weights of Female Mice Fed TNG

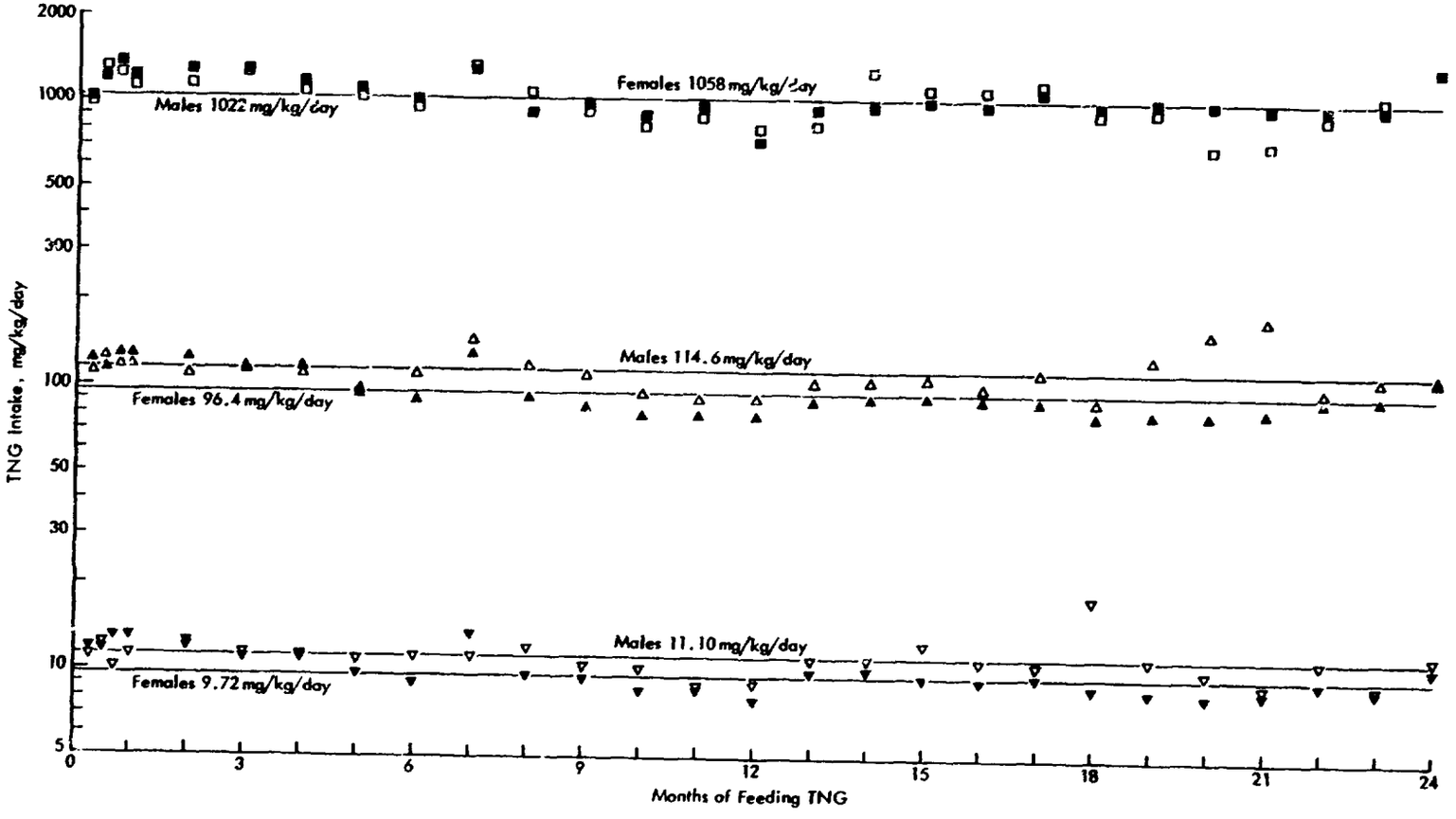

Figure 20 - Average Compound Intake of Male (open symbols) and Female (solid symbols) Mice Fed TNG

VI. GENERAL DISCUSSION AND CONCLUSIONS

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VI. GENERAL DISCUSSION AND CONCLUSIONS

The toxic doses and effects in all species tested, and the implications for human effects, are discussed below.

A. Toxic Doses

In dogs, there was a small, dose-related incidence of minimal methemoglobinemia after oral administration of 1, 5 and 25 mg/kg/day.

In rats, the low dose (3.04 mg/kg/day in feed for males and 3.99 mg/kg/day in feed for females) had no effects; the middle dose (31.5 and 38.1 mg/kg/day, respectively) had slight toxic effects and the high dose (363 and 434 mg/kg/day, respectively) was highly toxic, but not lethal.

Mice were less affected than rats. Both the low (11.1 mg/kg/day in feed for males and 9.7 mg/kg/day in feed for females) and middle (115 and 96 mg/kg/day, respectively) doses had no effect. The high dose (1020 and 1060 mg/kg/day, respectively) was toxic, but effects were not as widespread and severe as in the high-dose rats fed the same ration, even though the intake of TNG in mice was considerably larger.

Two similar studies were reported in the literature.[22,23] A total of 50 male and 48 female rats were given 0.03% TNG in their drinking water for 10 months, then observed for a further 8 months.[22] No carcinogenic effects were observed. These rats consumed 31 mg/kg/day, about the same as the middle-dose group in the present study. These results are compatible with ours. The companion study with mice[23] used doses up to 58.1 mg/kg/day, somewhat less than our middle dose. The only adverse effect seen with TNG was the occurrence of pituitary tumors in five females given the high dose of TNG, and in no other mice. These results are statistically significant ($p = 0.006$, exact analysis of contingency table for the females). We found pituitary tumors in only the low-dose mice (in one male and five females). It is probable that both of these results represent random variation, because of the inconsistency between the sexes and the lack of a dose-response relationship.

B. Target Organs

TNG had some non-specific effects on weight gain, feed consumption, and behavior. The target organs included the blood, liver and testis.

1. Non-Specific Effects

In the high-dose groups of both rats and mice there were decreases in weight gain and smaller decreases in feed consumption, especially in the first weeks of the study. After some months, it was apparent that these high-dose rodents were not grooming normally; their fur was rough, matted and a non-descript tan color. The mechanism of these effects is not known, but a mild sedation or central nervous system depression, either direct or due to hypoxia from the blood effects, could account for these observations.

The doses used did not significantly increase mortality. In fact, the large dose decreased mortality somewhat in the rats. The decreased weight gain could be a partial cause of this effect, since the high-dose rats were not emaciated. The weight difference was largely due to fat; the high-dose rats did not have the gross obesity characteristic of rats fed ad libitum.

2. The Blood

Methemoglobinemia was seen in all species. This was the only effect observed in dogs. This well-studied effect[14] is produced by most organic and inorganic nitrates and nitrites. These compounds, or probably, their nitrosamine and hydroxylamine reduction products, oxidize the iron in hemoglobin, producing methemeglobin. Within limits, the body can correct this effect. This was seen in dogs after large oral doses of TNG.[3] Inborn deficiencies in metabolism, such as glucose-6-phosphate dehydrogenase deficiency,[15] or high levels of the poison can overwhelm the normal protective measures, producing numerous secondary effects.

One common sequela of methemoglobinemia is the formation of aggregates of ill-defined degradation products in the erythrocytes, known as Heinz bodies. Heinz bodies are readily detected by staining and were seen in the rodents. Another sequela to excessive methemoglobinemia is anemia, due to destruction of degraded hemoglobin. In the present study, a "compensated anemia," a reticulocytosis with normal erythrocyte counts, occurred. The erythropoietic system increased production (as indicated by an increased proportion of immature cells, reticulocytes) enough to compensate for increased destruction. The high-dose rats sometimes had a higher erythrocyte count than the control rats. This probably was a hemoconcentration due to variation in body water, rather than the formed elements of the blood.

An unusual sequela of the methemoglobinemia seen in rats and mice was the occurrence of deposits of granules of pigment, typically golden-brown, in the liver, spleen and, occasionally, other organs. This pigment was not hemosiderin, because it did not react strongly to Prussian blue staining, but it is presumably a closely related degradation product of hemoglobin.

3. Liver

In almost all high-dose rats, there was hyperplasia of the bile ducts, often highly fibrous, known as cholangiofibrosis. In some rats, this was massive, resulting in extreme hepatomegaly.

Another effect seen in high-dose rats was the progressive development of hepatocellular carcinoma, a pattern seen with other chemicals[17,18] having completely different structures. The middle-dose rats had an increased incidence of the first stage of the progression (foci or areas of altered, hyperplastic hepatocytes), but most did not live long enough to develop the intermediate stage (neoplastic nodules), much less full-bloom carcinomas. This was the only adverse effect observed in the middle-dose rats.

4. Testis

High-dose male rats developed interstitial cell tumors of the testis. The pressure of the growing tumor on the seminiferous tubules produced a secondary sterility. A similar secondary sterility was apparent in the three-generation reproduction study because the testes had an increase in the interstitial tissue.

5. Spontaneous Tumors

In this strain (CD®) of rats, the most common spontaneous tumors are the pituitary adenoma and mammary tumors.[16] The high-dose rats had significant decreases in the incidence of both types of tumors. The mechanism is unknown. Since these tumors are the most frequent causes of unscheduled deaths, this decrease was probably the major cause of the increased longevity of the high-dose rats, especially the females.

C. Special Studies

No direct toxic effects were seen in the various special studies in rats including immunoglobin E assay, three generation-reproduction, mutagenesis and metabolism. However, indirect toxicity from the testicular effect and malnutrition was seen in the reproduction study.

D. Human Correlations

1. Toxic Effects

The toxic symptom normally expected from human occupational exposure[24] and therapeutic use[25] is a severe, throbbing headache due to dilation of the cranial blood vessels. These studies cannot provide direct evidence

of headache. However, behavioral studies[26] show some effects, particularly an aversion for TNG-dosed water, which could be explained by a TNG-induced headache. In addition, the decreased feed consumption observed in the present study during the first weeks could be a similar aversion. This aversion was, presumably, overcome by the well-known development of tolerance[24] or even physiological dependence[27] in the vasculature.

We could not find any reports of human toxicity other than those on the vasculature (headache, postural hypotension) and production of methemoglobinemia, nausea, vomiting and occasional hypersensitivity reactions. None of these observations seem related to the hepatic, testicular and tumor-reduction effects seen in rats.

2. Dose Relationship

Severe toxicity, including oncogenesis, was seen only in the high-dose rats, receiving about 400 mg/kg/day of TNG in the feed. This dose is about 3-1/2 orders of magnitude greater than the usual maximum human dose (10 mg/man/day or 0.14 mg/kg/day[21]). Furthermore, the usual dosage route in humans is sublingual.[24] Only a fraction of the human dose will be immediately delivered to the liver while in rats the portal circulation carries all the absorbed TNG to the liver. Thus, the dose delivered to the liver of the high-dose rats was at least 10,000-fold greater than the maximum dose delivered to human liver. Since minimal effects were seen in the middle-dose rats (35 mg/kg/day) and none in the low-dose rats (3.5 mg/kg/day), it is not surprising that no TNG-induced tumors have been reported in the human liver.

E. Water Quality Criteria

1. Rationale: Water quality criteria are used to estimate the amounts of noxious compounds in ambient water which will not be hazardous to the human population. The EPA has developed methodology[29] for the determination of these criteria. We will use our data on TNG to assess the risk to humans. Of the effects of TNG discussed above, the critical one is carcinogenicity.

As a matter of policy, the EPA uses the "one-hit model" to extrapolate animal carcinogenic data to man. This model, expressed mathematically as:

$$P = 1 - e^{-BD},$$

assumes that one molecule of a carcinogen delivered to the proper active site is adequate to initiate the irreversible process of carcinogenicity. Therefore, the probability (P) of an individual developing a tumor is a function of the dose (D) and the slope (B) of the dose-response curve, a measure of the potency of the subject carcinogen.

From the above chronic studies, the following data are available: nt, the number of animals exposed to the lowest dose that produced tumors at a level significantly higher than controls, using the Fischer exact test at the $p < 0.05$ level of significance; d, average dose per unit of time (mg/kg/day) during administration of the chemical; NT, the total number of animals exposed to the selected dose level; NC, the total number of control animals; nc, the number of control animals with the tumor type studied; Le, the maximum lifespan for the test animal (i.e., 6 weeks from birth to start of dosing, then 2 years of dosing); le, the actual maximum time of exposure for test animals; w, average weight of test animals in kg. These data are then converted to parameters applicable to humans, using the expanded model:

$$P_t = P_c + (1 - P_c) \cdot (1 - e^{-t^3 BD})$$

where P_t and P_c are the proportion of tumors in treated and control animals, respectively, and t is the ratio of test animal lifespan to human lifespan.

The human dose is considered to come from direct consumption of 2 liters of contaminated water each day and from the consumption of 0.0187 kg/day of fish (T) from the contaminated water. The TNG intake from the fish is derived from the bioconcentration factor (R) calculated by Mr. J. G. Pearson.[30/] From these data we can calculate the dose associated with a given P_t.

2. Calculations: The first step is determining the lowest dose group with a statistically significant tumor increase. These are listed in Table 100. Since we have effects in both sexes we calculate the criteria from the data set with the lowest dose - the males. The data used are summarized in Table 101.

3. Conclusion: Because TNG has carcinogenic effects, an ambient water concentration of zero is necessary for maximum protection of human health. However, exposure to 28.91 µg/liter for a lifetime produces an estimated risk of 10^{-5} (one in 100,000) that a tumor will develop in man.

A decrease of the concentration to 2.89 µg/liter would reduce risk to 10^{-6}, and further reduction to 0.29 µg/liter would reduce risk to 10^{-7}.

TABLE 100

SIGNIFICANT TUMOR INCIDENCES IN ANIMALS GIVEN TNG

Species, Sex	TNG Intake (mg/kg/day)	Tumor Type	Tumor Incidence		Probability[a/]
			Control	Treated	
Rat, Male	363	Hepatocellular carcinoma	0/24	13/21	0.000 002 8
Rat, Female	434	Hepatocellular carcinoma	0/29	11/25	0.000 047

a/ Fisher's exact test for contingency tables.

TABLE 101

DATA FOR WATER QUALITY CRITERION FOR TNG

Data:

nt	13
NT	21
nc	0
NC	24
Le (week)	110
le (week)	104
d (mg/kg/day)	363
w (kg)	0.530
L (week)	104
R	9.75
F (kg)	0.0187

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APPENDIX I

MANUAL FOR

HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

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HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

I. HEMATOLOGY AND CLINICAL LABORATORY TESTS

The usual blood sample from dogs is 8 ml, from monkeys 4 ml, and from rats 0.3 ml for hematology and about 8 ml for full analysis at termination.

A. Hematology

The following hematological analyses are performed on all blood samples from rats, dogs and monkeys.

1. Erythrocyte and leukocyte counts: A Coulter Electronic Particle Counter with 100 μ aperture is used.[1] Particle-free diluents (Isoton for RBC, Zap-Oglobin in Isoton for WBC, Coulter Electronics, Inc.) are counted to establish the background. Each blood sample is counted in duplicate. For each test day, two control blood samples (Diagnostic Technology, Inc.) are counted separately in duplicate.

2. Hematocrit: Hematocrit is determined in capillary tubes using a microcapillary centrifuge (International Equipment Company, Model MB). Two control blood samples (Diagnostic Technology, Inc.) are measured separately in duplicate.

3. Hemoglobin: Hemoglobin is measured as cyanomethemoglobin.[2] Each blood sample is measured in duplicate. Cyanomethemoglobin (Coulter Electronics, Inc.) is used as the standard. For each assay, two levels of the standard are used and two control blood samples (Diagnostic Technology, Inc.) are measured in duplicate.

4. Methemoglobin (Met-Hb): Met-Hb is measured by the method of Dubowski.[3] A positive control is made by adding potassium ferricyanide to control blood.

5. Heinz bodies: Heinz bodies are stained with methyl violet and the percent of Heinz bodies is calculated.

6. Mean corpuscular volume (MCV): MCV is calculated as follows:

$$\text{MCV } (\mu^3) = \frac{\text{Hematocrit x 10}}{\text{Erythrocytes in millions/mm}^3}$$

7. Mean corpuscular hemoglobin (MCHb): MCHb is calculated as follows:

$$\text{MCHb } (\mu\mu g) = \frac{\text{Hemoglobin (gm \%) x 10}}{\text{Erythrocytes in millions/mm}^3}$$

8. Mean corpuscular hemoglobin concentration (MCHbC): MCHbC is calculated as follows:

$$\text{MCHbC (gm \%)} = \frac{\text{Hemoglobin (gm \%) x 100}}{\text{Hematocrit}}$$

9. Differential leukocyte counts: Wright's stain is used to stain the leukocytes for examination.

10. Reticulocyte count: Reticulocytes are counted by the methylene blue method using the Miller disc.[4]

11. Platelet count: A Coulter Electronic Particle Counter with 70 μ aperture is used.[5] Particle-free Isoton is used as diluent and counted to establish the background. At weekly intervals, platelets are also visually counted in a hemocytometer with a phase microscope for comparison.[6]

12. Clotting time (dog and monkey): Clotting time is determined by the capillary tube procedure using two capillary tubes.[7] The time elapsed from the appearance of the blood from the animal and coagulation in either tube is measured.

B. Clinical Blood Tests

The following clinical blood chemistry tests are performed on all blood samples from dogs and monkeys and on blood samples from rats at termination.

1. Blood glucose: Fasting blood glucose is determined by Stein's hexokinase method.[8] Standard glucose solution (Dade) is used to establish a standard curve. For each assay, one level of the standard and two controls (Reference Serum, Worthington; and Validate, General Diagnostics) are measured.

2. Serum glutamic-oxaloacetic transaminase (SGOT): SGOT is measured by the method of Amador and Wacker.[9] Validate and Reference Serum are used as the enzyme reference for each assay.

3. Serum glutamic-pyruvic transaminase (SGPT): SGPT is measured by the method of Henry et al.[10] Validate and Reference Serum are used as the enzyme reference for each assay.

4. Alkaline phosphatase: Alkaline phosphatase is measured by the method of Bowers and McComb.[11] Validate and Reference Serum are used as the enzyme reference for each assay.

5. BUN: BUN is measured using the BUN Strate Kit (General Diagnostic) which is based on the urease method.[12] Three levels of Calibrate (General Diagnostics) are used to establish a standard curve. For each assay, two controls (Calibrate I and Validate) are used as the reference.

6. Creatinine: Creatinine is measured by a modified kinetic alkaline picrate procedure.[13] Creatinine Standard Solutions (Sigma Chemical Company) are used to establish a standard curve. For each assay, two levels of the standard and two controls (Calibrate I and Validate) are used as reference.

7. Lactate dehydrogenase (LDH): LDH is measured by the method of Wacker et al.[14] Precinorm E and Precipath E (Boehringer, Mannheim Corporation) are used as the enzyme controls for each assay.

8. α-Hydroxybutyrate dehydrogenase (α-HBDH): α HBDH is measured by the method of Rosalki and Wilkinson.[15] Precinorm E and Precipath E are used as the enzyme controls for each assay.

9. Creatine phosphokinase (CPK): CPK is measured by the improved procedure of Rosalki[16] based on the methods of Oliver.[17] Precinorm E and Precipath E are used as the enzyme controls for each assay.

C. Urinalysis

Urine samples are collected from animals before and during treatment as are the blood samples. The urine from rats is collected by slight manipulation of their body, and samples within each group are pooled. The monkeys and dogs are placed individually in metabolism cages, and urine is collected in the stainless steel pan. The urine from each dog and the pooled urine from rats are tested and examined for the following:

1. Protein: Urinary protein is determined with Labstix (Ames Company, Elkhart, Indiana).

2. Sugar: Urinary glucose and reducing substance are determined with Labstix (Ames Company).

3. Microscopic examination: Urine samples are centrifuged and the supernatant discarded. The residue is resuspended and examined microscopically for the presence of erythrocytes, leukocytes, epithelial cells, and crystals under high power field and for casts under low power field.

A positive urine control prepared with known amounts of protein and glucose in saline adjusted to pH 6.0 is run with each assay to check the reliability of the Labstix.

D. Occult Blood in Feces

Fecal samples are collected from animals before and during treatment as are the blood and urine samples. Occult blood in the feces is determined with Hematest Reagent Tablets (Ames Company, Elkhart, Indiana). A positive control (whole blood) and a negative control (distilled water) are included with each assay to check the reliability of the Hematest tablets.

E. Precision of Hematology and Clinical Blood Chemistry Tests

1. Reproducibility

For erythrocyte and leukocyte counts, hematocrit, hemoglobin, and the various clinical blood chemistry tests, the same control blood samples or control standards are used for day-to-day assays. The replication of results are excellent and are summarized in Table A.

The determination of differential leukocyte counts and reticulocyte counts are performed by experienced personnel. At weekly intervals, a blood sample is counted by two or more personnel to confirm the accuracy of the counting. Also at weekly intervals, the platelet counts obtained from a Coulter Electronic Particle Counter are compared with the direct visual counts in a hemocytometer using a phase microscope.

2. Reproducibility Within a Test Day

At monthly intervals, a blood sample is taken from a control dog and six or more determinations for erythrocyte, leukocyte, reticulocyte, and platelet counts, hemoglobin, and various clinical blood chemistry tests are performed to establish the reproducibility within an assay. The results are summarized in Table B.

3. Proficiency Test Service

We subscribe to the Proficiency Test Service of the Institute for Clinical Science, Hahnemann Medical College, Philadelphia, Pennsylvania (F. Wm. Sunderman, M.D., Director). On the first day of each month, this service sends two samples containing two different sera or solutions to all subscribers for measurements of one or more of the parameters usually analyzed in clinical laboratories. Participants report their results on a form furnished by the service. On the 15th day of the month, each participant receives a report from the service which includes: the results of a statistical analysis of the values reported by all the participating laboratories; a current review of pertinent methodology; a comprehensive bibliography; and validation of the results which the participating laboratory reported. This service enables each participating laboratory to obtain an unbiased and critical assessment of its proficiency in relation to that of 1,000 or so other clinical laboratories throughout the country. The service has been in continuous operation since 1949 and was given endorsement by the American Society of Clinical Pathologists in 1952 and by the Association of Clinical Scientists in 1957 and 1968. Our results have been found to be satisfactory and are summarized in Table C.

II. HISTOPATHOLOGY

A. Necropsy and Gross Examination

At termination or prior to imminent death, rats are killed with ether, and dogs and monkeys with an overdose of sodium pentobarbital. Animals that die on tests are kept refrigerated but not frozen until necropsy. The general physical condition and nutritional status of each animal at the time of death or termination are observed and recorded. Necropsy is performed as soon as possible after death. Gross changes of all tissues are carefully examined and recorded.

B. Organ Weights

The brain, liver, spleen, kidneys, adrenals, thyroids and gonads are trimmed free from surrounding tissues and weighed. The organ weight to body weight and/or brain weight ratios are then calculated.

C. Tissues for Microscopic Examination

Tissues to be examined include the eye, skin (breast), trachea, lung, tongue (except rat), salivary gland, liver, gallbladder (except rats), pancreas, esophagus, fundic and pyloric stomach, duodenum, jejunum, ileum, cecum, colon, kidneys, urinary bladder, gonads, and accessory organs, diaphragm and gracilis muscle, anterior pituitary, thyroids/parathyroids, adrenals, tonsil (except rat), thymus, spleen, prescapular (except rats) and mesenteric lymph nodes, rib bone with bone marrow, brain (sagittal section for rats; coronal sections of cerebral cortex, cerebellum, and brain stem for dog and monkey), spinal cord (lumbosacral plexus, dog and monkey), sciatic nerve and any other structures not mentioned which show abnormal gross changes.

D. Fixation and Staining of Tissues

All tissues are cut not to exceed 1 cm in thickness for fixation. For most tissues, neutral buffered 10% formalin is used. Sufficient volume of fixing solution is used and the tissues are changed to a fresh solution after 24 hours. The fixed tissues are processed in an Autotechnicon for dehydration, clearing, and infiltration and then embedded in paraffin. Routine H & E staining is used to stain the sectioned tissues for microscopic examination.

Supplementary tissue fixatives and staining techniques may be employed for more positive identification of special lesions such as calcification, pigments, fat deposition and other abnormal changes.

III. STATISTICAL ANALYSIS

Data are analyzed statistically using the Dunnett's multiple comparison procedure following an analysis of variance,[18] or our modification of this procedure for uneven numbers among groups. The chosen criterion significance is $p < 0.05$. The means of each group at various intervals during treatment are compared with pretreatment levels. For most experiments in beagles, three baseline (pretreatment) levels are obtained. The baseline levels for each animal are averaged and the mean is used in the analysis. In addition, the means of the various treated groups are compared with that of the control group at the respective time intervals.

IV. NORMAL VALUES

A. Hematology, Clinical Laboratory Tests and Bone Marrow

Since June 1971, we have used about 180 rhesus monkeys (Woodard Research Corporation, Herndon, Virginia, Primate Imports, Port Washington, New York, and PrimeLabs, Inc., Farmingdale, New Jersey) for various studies. The peripheral blood elements and clinical blood chemistry values of these monkeys before treatment and the myeloid/erythroid (M/E) ratio of the bone marrow of the monkeys used as normal controls varied among individual animals. The mean ± S.D. and the range of the various parameters for the males and females are summarized in Tables D and E, respectively.

Since September 1971, we have used about 525, 5 to 9 months old, beagles dogs (AKC registered, Hazelton Research Animals, Inc.). The peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow varied considerably among individual dogs. The mean ± S.D. and the ranges of the various parameters for the males and females are summarized in Tables H and I, respectively.

During the same period, we have used about 500, 7 to 10 weeks old, male albino rats (CD® Strain, Charles River Breeding Laboratories). As for the dogs, the individual variations of the peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow were large. The mean ± S.D. and the ranges of the various parameters for these male rats are summarized in Table L.

B. Absolute and Relative Organ Weights

Organ weights, both absolute and relative to body weight, of rhesus monkeys, beagle dogs, and albino rats are summarized in Tables F and G, J and K, and M, respectively. These were control animals used between June 1971 and December 1976.

C. Presence of Various Substances in the Urine

Various substances occasionally occurred in the urine of monkeys, dogs and rats. The results are summarized in Table N. Large percentage of urine samples from monkeys contained epithelial cells, i.e., 34.7% to 52.0%. Other substances occurred in 8.1% or less of the urine samples.

In dogs, protein, erythrocytes, leukocytes and epithelial cells were present in 19.1 to 21.6%, 16.5 to 19.8%, 22.6 to 24.6% or 24.7 to 25.7%, respectively, of the samples from dogs collected for analysis. Glucose,

crystals, and casts occurred in less than 2% of these samples. Some dogs had been bled and returned to the metabolism cages before the urine was removed for analysis. The high incidence of some of these substances in the urine of these dogs might be due to contamination with the fecal material and traces of blood dropped in the cage. Special care to avoid contamination has been undertaken.

In rats, large percentage of urine samples contained protein, i.e., 29.8 to 36.0%. A few samples contained erythrocytes, leukocytes, epithelial cells and crystals.

D. Occult Blood in the Feces

Less than 10% of the feces samples from monkeys or dogs was positive with the Hematest for occult blood. The results are summarized in Table O.

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TABLE A

REPRODUCIBILITY AMONG TEST DAYS ON THE SAME CONTROL SAMPLES OR STANDARDS[a/]

	No. of Determinations	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)			
Normal level	20	4.51 ± 0.07	4.36 - 4.67
Abnormal level	20	2.32 ± 0.04	2.25 - 2.40
Hematocrit (vol %)			
Normal level	20	44.3 ± 0.40	44 - 45
Abnormal level	20	22.8 ± 0.60	22 - 24
Hemoglobin (gm %)			
Normal level	20	14.2 ± 0.20	13.6 - 14.5
Abnormal level	20	7.4 ± 0.20	6.9 - 7.8
Leukocyte Counts (x 10^3/mm^3)			
Normal level	20	7.3 ± 0.50	6.8 - 8.7
Abnormal level	20	17.6 ± 0.80	16.3 - 18.7
Fasting Blood Glucose (mg %)	20	163.0 ± 7.5	151 - 178
SGOT (IU/ℓ)	23	61.7 ± 3.9	55 - 68
SGPT (IU/ℓ)	23	51.3 ± 2.6	46 - 55
Creatinine (mg %)	18	2.2 ± 0.3	1.6 - 2.6
BUN (mg %)	19	9.8 ± 0.2	9.5 - 10.2
Bilirubin (mg %)	11	0.8 ± 0.1	0.8 - 1.0
Alkaline Phosphatase (IU/ℓ)	22	71.6 ± 5.4	62 - 80
CPK	11	153.0 ± 7.7	139 - 161
LDH	8	98.0 ± 2.4	95 - 101
HBDH	8	226.0 ± 7.2	214 - 238

a/ Performed in December 1976.

TABLE B

REPRODUCIBILITY WITHIN A TEST DAY ON THE SAME SPECIMEN[a]

	Mean ± S.D.[b]	Range
Erythrocytes (x 10^6/mm^3)	5.90 ± 0.14	5.73 - 6.08
Reticulocytes (%)	0.63 ± 0.12	0.44 - 0.79
Hematocrit (vol %)	46.8 ± 0.6	46.0 - 47.5
Hemoglobin (gm %)	16.1 ± 0.2	15.8 - 16.1
Platelets (x 10^5/mm^3)	1.56 ± 0.07	1.49 - 1.66
Leukocytes (x 10^3/mm^3)	10.8 ± 0.4	10.2 - 11.3
Bands (%)	0 ± 0	0 - 0
Neutrophils (%)	64.3 ± 3.1	61 - 69
Lymphocytes (%)	29.0 ± 4.9	23 - 35
Eosinophils (%)	3.2 ± 0.8	2 - 4
Basophils (%)	0 ± 0	0 - 0
Monocytes (%)	3.4 ± 0.9	3 - 5
Atypical (%)	0 ± 0	0 - 0
Nucleated RBC (%)	0 ± 0	0 - 0
Methemoglobin (gm %)	0 ± 0	0 - 0
Fasting Glucose (mg %)	96.7 ± 3.0	32 - 101
SGOT (IU/ℓ)	23.2 ± 2.8	21 - 28
SGPT (IU/ℓ)	25.3 ± 2.1	24 - 28
Creatinine (mg %)	0.6 ± 0.1	0.5 - 0.6
BUN (mg %)	9.0 ± 0.0	9 - 9
Alkaline Phosphatase (IU/ℓ)	63.5 ± 1.1	62 - 65
CPK	44.0 ± 1.6	43 - 46
LDH	38.5 ± 1.6	37 - 40
HBDH	42.0 ± 1.6	40 - 43

a/ Performed in October 1976.
b/ Six determinations from an adult beagle blood sample.

TABLE C

PROFICIENCY TEST SERVICE (PTS) REPORTS (1975-1976)[a/]

Unknowns	MRI Results	PTS Results	Participating Laboratories (10-90 Percentiles) Median	Mean	Acceptable Performance[b/]
Hemoglobin	13.8 gm %	13.8	13.8	13.8	13.6 - 14.0
	18.1 gm %	17.9	17.9	17.8	17.6 - 18.2
Serum Protein	6.6 mg %	7.1	7.0	7.0	6.7 - 7.3
Fasting Glucose	272.0 mg %	264.5	266.0	263.0	240 - 290
	229.0 mg %	221.4	220.5	222.5	200 - 240
BUN	12.1 mg %	12.0	12.0	12.2	11.0 - 13.0
	38.4 mg %	40.1	40.3	39.2	36.0 - 44.0
Creatinine	1.0 mg %	1.0	1.0	1.0	0.8 - 1.3
	4.3 mg %	4.4	4.5	4.4	3.9 - 4.9
Bilirubin	3.9 mg %	4.16	4.15	4.14	3.5 - 4.6
	1.3 mg %	1.78	1.80	1.77	1.5 - 2.1
Cholesterol	175.0 mg %	161.4	161.0	162.0	145 - 175
	100.0 mg %	109.8	109.4	111.0	98 - 120
Ca	15.7 meq/ℓ	15.4	15.4	15.3	14.1 - 16.4
	9.5 meq/ℓ	9.8	9.8	9.8	9.2 - 10.3
Na	156.0 meq/ℓ	155.8	156.0	155.5	153 - 158
K	7.3 meq/ℓ	7.5	7.5	7.5	7.3 - 7.7
Cl	96.0 meq/ℓ	97.8	98.0	97.5	96 - 101
	78.0 meq/ℓ	79.4	79.0	80.0	77 - 83
Mg	1.0 meq/ℓ	1.1	1.1	1.2	0.9 - 1.4
	1.9 meq/ℓ	2.0	2.0	2.1	1.8 - 2.3

a/ To date, we have received unknowns for phosphorus, uric acid, and serum iron. We do not routinely perform these determinations.

b/ Based on values submitted by participants by 10th of month.

TABLE D

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE RHESUS MONKEYS[a]

	Male Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x $10^6/mm^3$)	108	3.74 ± 0.50	5.51 ± 0.45	3.75 - 6.61
Reticulocytes (%)	108	3.74 ± 0.50	0.97 ± 0.42	0.07 - 2.41
Hematocrit (vol %)	108	3.74 ± 0.50	43.0 ± 2.6	37.0 - 50.0
Hemoglobin (gm %)	108	3.74 ± 0.50	13.4 ± 0.8	10.8 - 15.4
MCV (μ^3)	108	3.74 ± 0.50	77.8 ± 7.0	69.6 - 117.3
MCHb ($\mu\mu g$)	108	3.74 ± 0.50	24.4 ± 1.8	21.0 - 33.6
MCHbC (mg %)	108	3.74 ± 0.50	31.4 ± 1.3	27.2 - 34.1
Platelets (x $10^5/mm^3$)	99	3.74 ± 0.50	3.08 ± 0.45	0.80 - 7.10
Leukocytes (x $10^3/mm^3$)	108	3.74 ± 0.50	10.4 ± 4.9	3.8 - 30.1
Neutrophils I (%)	108	3.74 ± 0.50	0.18 ± 0.45	0 - 2
Neutrophils M (%)	108	3.74 ± 0.50	39.30 ± 17.72	10 - 83
Lymphocytes (%)	108	3.74 ± 0.50	56.83 ± 17.74	13 - 84
Eosinophils (%)	108	3.74 ± 0.50	1.91 ± 2.42	0 - 13
Monophils (%)	108	3.74 ± 0.50	1.37 ± 1.58	0 - 7
Basophils (%)	108	3.74 ± 0.50	0.04 ± 0.20	0 - 2
Atypical cells (%)	108	3.74 ± 0.50	0.00 ± 0.00	0 - 0
Nucleated RBC (%)		3.74 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	100	3.76 ± 0.51	96.9 ± 15.2	59 - 127
SGOT (IU/ℓ)	100	3.76 ± 0.51	33.7 ± 9.2	20 - 60
SGPT (IU/ℓ)	100	3.76 ± 0.51	31.3 ± 7.8	15 - 46
Alkaline Phosphatase (IU/ℓ)	100	3.76 ± 0.51	360.0 ± 116.0	143 - 501
BUN (mg %)	100	3.76 ± 0.51	19.5 ± 7.5	12 - 65
Proth. Time (sec)	62	3.91 ± 0.44	10.2 ± 0.7	9.3 - 11.9
Serum Creat. (mg %)	100	3.76 ± 0.51	1.1 ± 0.3	0.6 - 1.8
Bilirubin				
Total (mg %)	62	3.91 ± 0.44	0.1 ± 0.2	0.0 - 0.8
Direct (mg %)	62	3.91 ± 0.44	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	62	3.91 ± 0.44	18.0 ± 7.4	2 - 34
Na (mEq/ℓ)	62	3.91 ± 0.44	154.0 ± 19.1	144 - 179
K (mEq/ℓ)	62	3.91 ± 0.44	4.8 ± 0.6	3.9 - 5.7
Cl (mEq/ℓ)	62	3.91 ± 0.44	109.0 ± 6.4	93 - 118
Ca (mEq/ℓ)	62	3.91 ± 0.44	5.2 ± 0.4	4.2 - 6.3
Mg (mEq/ℓ)	62	3.91 ± 0.44	1.6 ± 0.1	1.2 - 1.8
Bone Marrow				
Myeloid/erythroid ratio	15	3.65 ± 0.41	1.5 ± 0.3	1.5 - 2.1

a/ Data collected between June 1971 and December 1976.

TABLE E

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE RHESUS MONKEYS[a/]

	Female Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	81	3.51 ± 0.48	5.33 ± 0.40	4.25 - 6.03
Reticulocytes (%)	81	3.51 ± 0.48	1.07 ± 0.54	0.35 - 3.31
Hematocrit (vol %)	81	3.51 ± 0.48	41.5 ± 2.8	30.0 - 46.0
Hemoglobin (gm %)	81	3.51 ± 0.48	13.1 ± 1.0	7.9 - 14.1
MCV (μ^3)	81	3.51 ± 0.48	77.7 ± 5.3	66.5 - 95.2
MCHb ($\mu\mu$g)	81	3.51 ± 0.48	24.6 ± 1.7	17.6 - 29.7
MCHbC (mg %)	81	3.51 ± 0.48	31.6 ± 1.4	26.6 - 34.2
Platelets (x 10^5/mm^3)	81	3.51 ± 0.48	3.11 ± 1.23	1.85 - 7.90
Leukocytes (x 10^3/mm^3)	81	3.51 ± 0.48	9.5 ± 3.9	3.2 - 24.8
Neutrophils I (%)	81	3.51 ± 0.48	0.10 ± 0.43	0 - 3
Neutrophils M (%)	81	3.51 ± 0.48	36.41 ± 13.32	13 - 56
Lymphocytes (%)	81	3.51 ± 0.48	60.38 ± 13.26	41 - 79
Eosinophils (%)	81	3.51 ± 0.48	2.28 ± 3.10	0 - 18
Monophils (%)	81	3.51 ± 0.48	0.75 ± 0.98	0 - 4
Basophils (%)	81	3.51 ± 0.48	0.03 ± 0.22	0 - 1
Atypical cells (%)	81	3.51 ± 0.48	0.00 ± 0.00	0 - 0
Nucleated RBC (%)	74	3.56 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	81	3.51 ± 0.48	92.1 ± 15.3	57 - 116
SGOT (IU/ℓ)	81	3.51 ± 0.48	32.1 ± 7.6	20 - 70
SGPT (IU/ℓ)	81	3.51 ± 0.48	30.1 ± 7.6	12 - 39
Alkaline Phosphatase (IU/ℓ)	81	3.51 ± 0.48	349.9 ± 112.3	148 - 572
BUN (mg %)	81	3.51 ± 0.48	17.3 ± 4.2	13 - 29
Proth. Time (sec)	59	3.56 ± 0.43	10.5 ± 0.9	9.7 - 12.3
Serum Creat. (mg %)	81	3.51 ± 0.48	1.1 ± 0.3	0.6 - 1.7
Bilirubin				
Total (mg %)	81	3.51 ± 0.48	0.1 ± 0.1	0.0 - 0.8
Direct (mg %)	81	3.51 ± 0.48	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	59	3.56 ± 0.43	16.4 ± 8.3	5 - 34
Na (mEq/ℓ)	59	3.56 ± 0.43	158.2 ± 6.5	147 - 174
K (mEq/ℓ)	59	3.56 ± 0.43	4.8 ± 0.7	3.9 - 6.2
Cl (mEq/ℓ)	59	3.56 ± 0.43	109.0 ± 6.1	95 - 113
Ca (mEq/ℓ)	59	3.56 ± 0.43	5.3 ± 0.5	4.3 - 6.3
Mg (mEq/ℓ)	59	3.56 ± 0.43	1.6 ± 0.2	1.3 - 2.0
Bone Marrow				
Myeloid/erythroid ratio	11	3.49 ± 0.62	1.4 ± 0.3	1.0 - 1.8

a/ Data collected between June 1971 and December 1976.

TABLE F

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	82 ± 17	64 - 122
Spleen (gm)	4.6 ± 1.8	2.0 - 9.3
Kidneys (gm)	15.1 ± 3.8	8.0 - 22.0
Adrenals (gm)	0.73 ± 0.15	0.45 - 0.86
Thyroids (gm)	0.57 ± 1.30	0.37 - 0.81
Testes (gm)	1.29 ± 0.67	0.53 - 3.30
	Relative (per kg body weight) Mean ± S.D.	Range
Liver (gm)	23.4 ± 2.5	18.8 - 30.4
Spleen (gm)	1.25 ± 0.47	0.57 - 2.38
Kidneys (gm)	4.13 ± 0.92	2.20 - 6.43
Adrenals (mg)	201 ± 44	129 - 254
Thyroids (mg)	154 ± 42	86 - 250
Testes (gm)	0.34 ± 0.11	0.18 - 0.53

a/ Data collected between September 1971 and December 1976 from 17 monkeys weighing 3.71 ± 0.48 kg, used as control animals.

TABLE G

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	83 ± 17	64 - 122
Spleen (gm)	3.8 ± 1.4	2.0 - 6.0
Kidneys (gm)	14.5 ± 2.8	11.0 - 20.0
Adrenals (gm)	0.68 ± 0.16	0.53 - 1.14
Thyroids (gm)	0.60 ± 0.20	0.37 - 1.11
Ovaries (gm)	0.28 ± 0.10	0.14 - 0.45
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	25.4 ± 5.8	19.2 - 37.4
Spleen (gm)	1.16 ± 0.49	0.60 - 1.89
Kidneys (gm)	4.40 ± 0.86	3.20 - 6.25
Adrenals (mg)	212 ± 80	138 - 438
Thyroids (mg)	173 ± 66	97 - 346
Ovaries (mg)	82 ± 28	43 - 140

a/ Data collected between September 1971 and December 1976 from 11 monkeys weighing 3.39 ± 0.58 kg, used as controls.

TABLE H

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE BEAGLE DOGS[a/]

	Male Beagle Dogs				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	276	4 - 7	8.3 ± 1.7	5.55 ± 0.73	3.62 - 7.60
Reticulocytes (%)	284	4 - 7	8.3 ± 1.7	0.72 ± 0.46	0.04 - 4.25
Hematocrit (vol %)	276	4 - 7	8.3 ± 1.7	41.6 ± 3.5	31 - 50
Hemoglobin (gm %)	276	4 - 7	8.3 ± 1.7	13.5 ± 1.4	10.0 - 16 9
MCV (μ^3)	276	4 - 7	8.3 ± 1.7	75.6 ± 8.3	56.7 - 127.1
MCHb ($\mu\mu$g)	276	4 - 7	8.3 ± 1.7	24.6 ± 3.0	17.1 - 41.7
MCHbC (mg %)	276	4 - 7	8.3 ± 1.7	32.5 ± 1.5	28.1 - 40.3
Platelets (x 10^5/mm^3)	270	4 - 7	8.4 ± 1.7	2.91 ± 1.02	0.93 - 6.35
Leukocytes (x 10^3/mm^3)	284	4 - 7	8.3 ± 1.7	11.9 ± 3.5	4.6 - 24.6
Neutrophils I (%)	284	4 - 7	8.3 ± 1.7	0.55 ± 1.06	0 - 6
Neutrophils M (%)	284	4 - 7	8.3 ± 1.7	56.81 ± 9.47	22 - 80
Lymphocytes (%)	284	4 - 7	8.3 ± 1.7	37.94 ± 9.26	13 - 71
Eosinophils (%)	284	4 - 7	8.3 ± 1.7	2.76 ± 2.93	0 - 16
Monophils (%)	284	4 - 7	8.3 ± 1.7	1.78 ± 1.84	0 - 11
Basophils (%)	284	4 - 7	8.3 ± 1.7	0.01 ± 0.10	0 - 2
Atypical cells (%)	284	4 - 7	8.3 ± 1.7	0.11 ± 0.37	0 - 2
Nucleated RBC (%)	284	4 - 7	8.3 ± 1.7	0.02 ± 0.10	0 - 2
Fasting Glucose (mg %)	284	4 - 7	8.3 ± 1.7	100.9 ± 12.6	66 - 134
SGOT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	23.2 ± 7.4	11 - 59
SGPT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	25.7 ± 7.9	8 - 46
Alkaline Phosphatase (IU/ℓ)	276	4 - 7	8.3 ± 1.7	73.3 ± 18.5	21 - 133
BUN (mg %)	284	4 - 7	8.3 ± 1.7	12.1 ± 3.3	4 - 23
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	9.4 ± 1.6	1.6 ± 0.4	1.1 - 3.0

a/ Data collected between September 1971 and December 1976.

TABLE I

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE BEAGLE DOGS[a/]

	Female Beagle Dogs			Observed Results	
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	257	4 - 7	6.9 ± 1.3	5.59 ± 0.73	3.27 - 7.75
Reticulocytes (%)	265	4 - 7	6.9 ± 1.3	0.74 ± 0.52	0.04 - 5.05
Hematocrit (vol %)	257	4 - 7	6.9 ± 1.3	42.3 ± 3.5	32 - 51
Hemoglobin (gm %)	257	4 - 7	6.9 ± 1.3	13.7 ± 1.3	11.0 - 18.6
MCV (μ^3)	257	4 - 7	6.9 ± 1.3	76.7 ± 9.7	55.8 - 128.4
MCHb ($\mu\mu$g)	257	4 - 7	6.9 ± 1.3	24.8 ± 3.3	17.1 - 41.6
MCHbC (mg %)	257	4 - 7	6.9 ± 1.3	32.3 ± 1.6	28.7 - 40.4
Platelets (x 10^5/mm^3)	227	4 - 7	6.9 ± 1.3	3.08 ± 1.15	1.06 - 7.95
Leukocytes (x 10^3/mm^3)	265	4 - 7	6.9 ± 1.3	10.9 ± 3.4	3.8 - 26.9
Neutrophils I (%)	265	4 - 7	6.9 ± 1.3	0.54 ± 1.16	0 - 7
Neutrophils M (%)	265	4 - 7	6.9 ± 1.3	57.08 ± 10.10	31 - 85
Lymphocytes (%)	265	4 - 7	6.9 ± 1.3	37.15 ± 10.46	10 - 61
Eosinophils (%)	265	4 - 7	6.9 ± 1.3	2.37 ± 2.25	0 - 13
Monophils (%)	265	4 - 7	6.9 ± 1.3	1.94 ± 2.01	0 - 9
Basophils (%)	265	4 - 7	6.9 ± 1.3	0.01 ± 0.09	0 - 1
Atypical cells (%)	265	4 - 7	6.9 ± 1.3	0.11 ± 0.43	0 - 4
Nucleated RBC (%)	265	4 - 7	6.9 ± 1.3	0.03 ± 0.17	0 - 2
Fasting Glucose (mg %)	248	4 - 7	6.9 ± 1.3	99.6 ± 14.4	55 - 130
SGOT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	23.5 ± 7.2	6 - 52
SGPT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	25.3 ± 7.0	8 - 49
Alkaline Phosphatase (IU/ℓ)	257	4 - 7	6.9 ± 1.3	73.5 ± 19.2	30 - 146
BUN (mg %)	265	4 - 7	6.9 ± 1.3	12.4 ± 3.3	4 - 26
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	7.8 ± 1.4	1.4 ± 0.3	1.1 - 2.4

a/ Data collected between September 1971 and December 1976.

TABLE J

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	264 ± 51	166 - 384
Spleen (gm)	58 ± 25	22 - 167
Kidneys (gm)	53 ± 10	32 - 71
Adrenals (gm)	1.12 ± 0.26	0.74 - 1.75
Thyroids (gm)	1.03 ± 0.32	0.55 - 2.50
Testes (gm)	6.60 ± 4.36	1.32 - 18.00
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	27.9 ± 4.2	19.6 - 42.3
Spleen (gm)	6.0 ± 2.0	2.8 - 12.5
Kidneys (gm)	5.6 ± 0.8	4.0 - 7.7
Adrenals (mg)	117 ± 25	70 - 165
Thyroids (mg)	108 ± 34	56 - 211
Testes (gm)	0.67 ± 0.39	0.13 - 1.67

a/ Data collected between September 1971 and December 1976 from 51 dogs, weighing 9.3 ± 1.8 kg, used as control animals.

TABLE K

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	218 ± 51	106 - 322
Spleen (gm)	48 ± 21	16 - 103
Kidneys (gm)	43 ± 9	24 - 71
Adrenals (gm)	1.04 ± 0.26	0.49 - 1.65
Thyroids (gm)	0.88 ± 0.25	0.55 - 1.91
Ovaries (gm)	0.74 ± 0.24	0.38 - 1.27
	Relative (per kg body weight) Mean ± S.D.	Range
Liver (gm)	28.2 ± 5.0	20.7 - 38.8
Spleen (gm)	6.0 ± 2.3	3.1 - 10.9
Kidneys (gm)	5.5 ± 0.9	3.7 - 7.9
Adrenals (mg)	135 ± 35	67 - 215
Thyroids (mg)	112 ± 31	75 - 219
Ovaries (mg)	96 ± 33	54 - 222

a/ Data collected between September 1971 and December 1976 from 49 dogs, weighing 7.7 ± 1.5 kg, used as control animals.

TABLE L

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE ALBINO RATS[a/]

	Male Rats				
	Number Studied	Age (weeks)	Body Weight (gm) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	527	5 - 7	168 ± 22	5.84 ± 0.54	3.24 - 7.60
Reticulocytes (%)	461	5 - 7		3.04 ± 1.80	0.30 - 6.83
Hematocrit (vol %)	525	5 - 7	168 ± 22	45.1 ± 3.2	40 - 58
Hemoglobin (gm %)	525	5 - 7	168 ± 22	13.7 ± 0.9	11.8 - 17.1
MCV (μ^3)	525	5 - 7	168 ± 22	78.1 ± 16.3	62.3 - 104.6
MCHb ($\mu\mu$g)	525	5 - 7	168 ± 22	23.7 ± 2.6	19.2 - 41.0
MCHbC (mg %)	525	5 - 7	168 ± 22	30.5 ± 1.8	21.1 - 36.9
Platelets (x 10^5/mm^3)	473	5 - 7	164 ± 24	4.93 ± 1.23	2.30 - 7.95
Leukocytes (x 10^3/mm^3)	448	5 - 7	164 ± 24	15.4 ± 4.0	6.3 - 20.8
Neutrophils I (%)	448	5 - 7	164 ± 24	0.07 ± 0.31	0 - 3
Neutrophils M (%)	448	5 - 7	164 ± 24	14.1 ± 6.2	4 - 29
Lymphocytes (%)	448	5 - 7	164 ± 24	83.63 ± 6.75	52 - 96
Eosinophils (%)	448	5 - 7	164 ± 24	0.64 ± 0.91	0 - 6
Monophils (%)	448	5 - 7	164 ± 24	1.23 ± 1.73	0 - 13
Basophils (%)	448	5 - 7	164 ± 24	0.01 ± 0.15	0 - 2
Atypical cells (%)	448	5 - 7	164 ± 24	0.01 ± 0.12	0 - 2
Nucleated RBC (%)	448	5 - 7	164 ± 24	0.10 ± 0.42	0 - 4
Fasting Glucose (mg %)	125	10 - 12	348 ± 72	130.9 ± 17.2	94 - 165
SGOT (IU/ℓ)	125	10 - 12	348 ± 72	108.2 ± 34.5	63 - 223
SGPT (IU/ℓ)	125	10 - 12	348 ± 72	34.2 ± 16.5	17 - 120
Alkaline Phosphatase (IU/ℓ)	125	10 - 12	348 ± 72	94.9 ± 30.0	32 - 153
BUN (mg %)	125	10 - 12	348 ± 72	16.4 ± 4.7	8 - 41
Bone Marrow					
Myeloid/erythroid ratio	109	10 - 12	349 ± 63	1.7 ± 0.5	1.0 - 2.6

a/ Data collected between September 1971 and December 1976.

TABLE M

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE ALBINO RATS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	10.89 ± 2.87	7.18 - 15.09
Spleen (gm)	0.65 ± 0.11	0.34 - 0.89
Kidneys (gm)	2.64 ± 0.37	1.84 - 3.58
Adrenals (mg)	63.6 ± 9.5	21.9 - 73.5
Thyroids (mg)	26.3 ± 5.8	14.3 - 37.7
Testes (gm)	2.98 ± 0.51	1.76 - 3.81
	Relative (per 100 gm body weight) Mean ± S.D.	Range
Liver (gm)	2.96 ± 0.42	2.09 - 4.01
Spleen (gm)	0.19 ± 0.08	0.10 - 0.30
Kidneys (gm)	0.76 ± 0.10	0.22 - 0.88
Adrenals (mg)	18.6 ± 5.8	5.8 - 22.4
Thyroids (mg)	7.6 ± 2.7	4.2 - 12.7
Testes (gm)	0.87 ± 0.15	0.23 - 1.09

a/ Data collected between September 1971 and December 1976 from 139 rats, weighing 352 ± 59 gm, used as control animals.

TABLE N

PRESENCE OF VARIOUS SUBSTANCES IN THE URINE OF MALE AND FEMALE MONKEYS, DOGS AND MALE RATS

Species:		Monkeys		Dogs		Rats[a]	
No. of Animals:		141[b]	18	615[b]	112	84[b]	18
No. of Collections:		141	98[c]	615	565[c]	84	56[d]
Glucose:	< 250 mg %	0[e]	2.0 (2)	0.2 (1)	0.7 (4)	0	0
	> 250 mg %	0	0	0.5 (3)	0.2 (1)	0	0
Protein:	< 100 mg %	3.5 (5)	6.1 (6)	19.3 (119)	17.3 (98)	29.8 (25)	36.0 (18)
	> 100 mg %	0	2.0 (2)	2.3 (14)	1.8 (10)	0	0
RBC:[f]	Moderate	1.4 (2)	3.1 (3)	16.4 (101)	13.3 (75)	3.6 (3)	8.0 (4)
	Excessive	0	0	3.4 (21)	3.2 (18)	0	0
WBC:[f]	Moderate	1.4 (2)	2.0 (2)	18.7 (115)	20.9 (118)	0	4.0 (2)
	Excessive	0	0	3.9 (24)	3.7 (21)	0	0
Epithelium:[g]	Moderate	31.2 (44)	44.9 (44)	21.0 (129)	21.9 (124)	0	8.0 (4)
	Excessive	3.5 (5)	7.1 (7)	4.7 (29)	2.8 (16)	0	0
Crystal:[h]	Moderate	0.7 (1)	2.0 (2)	0.2 (1)	0.7 (4)	0	2.0 (1)
	Excessive	0	0	0.2 (1)	0.7 (4)	0	2.0 (1)
Casts:	Positive	0.7 (1)	5.1 (5)	0	0.9 (5)	0	0

a/ Pooled sample of 4-20 rats.
b/ Baseline data collected from all animals employed between September 1971 and December 1976.
c/ Data collected at weekly intervals for 4-7 collections from controls employed between September 1971 and December 1976.
d/ Data collected at 2-week intervals for 2-4 collections from control rats employed between September 1971 and December 1976.
e/ Percent of total (number of samples).
f/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/field (x 440).
g/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, > 25 cells/field (x 100).
h/ Normal, none; moderate, 1-5 crystals; excessive, > 5 crystals/field (x 100).

TABLE O

PRESENCE OF OCCULT BLOOD IN THE FECES OF MALE AND FEMALE MONKEYS AND DOGS

Species:		Monkeys		Dogs	
No. of Animals:		44[a]	8	118[a]	30
No. of Collections:		44	48[b]	118	156[b]
Occult Blood:	Negative	90.9 (40)[c]	95.8 (46)	94.1 (111)	91.7 (143)
	Positive	9.1 (4)	4.2 (2)	5.9 (7)	8.3 (13)

a/ Baseline data collected from all animals employed between July 1974 and December 1976.

b/ Data collected at weekly intervals for 4-7 collections from controls employed between July 1974 and December 1976.

c/ Percent of total (number of samples).

APPENDIX II

MANUAL FOR

STUDY OF DEVELOPMENTAL TOXICITY

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STUDY OF DEVELOPMENTAL TOXICITY

I. INTRODUCTION

The thalidomide catastrophe provides an unfortunate example of the need for reliable information concerning the effects of agents on human development. Prospective and retrospective epidemiological studies are the only ethical procedures currently available to obtain this information in humans. There are, however, a variety of protocols available to obtain preliminary developmental toxicity information in animals. This preliminiary animal information can be used to form the basis from which it is possible to evaluate the risk of exposing the human population to potentially toxic agents.

The purpose of this manual is to describe the protocol used in our laboratory to obtain developmental toxicity information. Sections, in addition, are included which discuss both the statistical analysis and interpretation of the data. A working definition of common anomalies is presented. These studies are based on "The Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use" distributed by the U.S. Food and Drug Administration, 1966, and "The Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity" distributed by the Ministry of Health and Welfare, Canada, 1973.

II. PROTOCOL FOR STUDY OF DEVELOPMENTAL TOXICITY

A. Fertility and General Reproductive Performance Study

1. Objectives

The emphasis in this phase is placed on determining the effect of an agent on gonadal function, estrus cycle, mating behavior, conception rates, and the early stages of development. This study serves as an overall pilot screening of the agent on the entire reproductive process including organogenesis, late stages of gestation, parturition, and lactation. The results obtained from this phase serve as a guide for conducting subsequent studies in greater depth.

2. Method

The rat is the animal generally used for this study and both males and females are used to provide an adequate study of fertility. Male rats,

at least 40 days of age, are treated for 60 to 80 days prior to mating to determine if the agent affects spermatogenesis. Male animals from subacute or chronic toxicity studies may be used and each male, from a group of at least 10 animals, is bred with two non-treated females. Each male is exposed overnight to females in proestrus or early estrus until (1) a male mates with two females or (2) a male is exposed, on at least three different occasions, to a total of at least five receptive females. A female is considered receptive if there is an estrous vaginal smear the morning following exposure. This procedure minimizes attributing male infertility to sexual inexperience.

Sexually mature female rats are treated for at least 14 days prior to mating with untreated males. The stages of the estrous cycle are determined by vaginal smears to verify that the animals cycle normally and to detect possible treatment effects on the duration of the estrous cycle. The occurrence of copulation is established by daily vaginal inspection for the presence of sperm. The day on which evidence of copulation is discovered is identified as being day 0 of gestation. Confirmation of pregnancy, however, is not obtained until the animal is sacrificed on day 13 of gestation or delivers a litter at the end of gestation. Females treated prior to mating are continued on the same treatment schedule until the time of sacrifice.

Half of the females from each group are sacrificed on day 13 of gestation. The dams are examined for number of corpora lutea and implantation sites, number and distribution of embryos in each uterine horn, presence of empty implantation sites, embryos undergoing resorption, and any abnormal conditions. The following parameters are determined:

a. Number of viable litters (litters with one or more viable implants)
b. Corpora lutea/dam
c. Total implants/dam
d. Viable implants/dam
e. Indexes of
    (1) Fertility: confirmed pregnancies/sperm positive females
    (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
    (3) Implantation: implants/corpora lutea
    (4) Implant viability: viable fetuses/implants

The remaining dams are allowed to deliver and the litters are examined at birth, day 4, and day 21. The litters are examined for number, weight, mortality, and abnormalities of the pups. The following parameters are determined:

a. Number of viable litters (litters with one or more viable pups)
b. Pups/dam
c. Weight of pups
d. Indexes of
   (1) Fertility: confirmed pregnancies/sperm positive females
   (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
   (3) Implant viability: viable pups/implants
   (4) Viability: pups alive at day 4/pups alive at birth
   (5) Lactation: pups alive at day 21/pups alive at day 4

B. Teratology Study

1. Objectives

The objective of this phase is to determine if an agent has a potential for producing embryotoxicity and/or teratogenicity. Treatment, therefore, is restricted to the period of organogenesis. Dosage may be high during this brief treatment period in order to obtain results concerning teratogenic potential and risk.

2. Method

Two species of animals are employed in this test. The species most frequently used are the mouse, rat, and rabbit. Drug treatment covers the period of organogenesis which is day 6 through 15 of gestation for the mouse and rat and day 6 through 18 for the rabbit.

Sexually mature virgin mice are obtained from reputable suppliers and conditioned in our animal quarters for 10 days. The conditioning period permits the animals to stabilize and establish regular estrus cycles of 4 to 5 days in duration. Females are placed overnight with a non-treated proven male breeder and examined the next morning for evidence of copulation. Successful mating is identified by the presence of a vaginal copulatory plug. The day that plugs are discovered is identified as day 0 of gestation. Mice are sacrificed on day 18 of gestation for fetal examination.

Sexually mature virgin female rats are obtained and conditioned as previously described for mice. Females are examined by vaginal lavage late in the afternoon for signs of proestrus (75-90% of nucleated epithlial cells). Females in proestrus are placed overnight with an experienced male. The following morning, females are examined for sperm or the presence of a vaginal

plug. The plug, however, is not as reliable an indicator of successful mating in rats as it is in mice. Rats are sacrificed on day 20 of gestation and examined for fetal anomalies.

Virgin female rabbits, 6 to 8 months of age, are obtained from commercial sources and are conditioned for 18 days in our animal quarters. Ovulation is induced by the intravenous administration of 1 mg/kg pituitary lutenizing hormone. Females are artificially inseminated within 1 hour by the method of Gibson, et al.[1] Fetuses are delivered by cesarean section on days 27 to 28 of pregnancy and examined for anomalies.

Mouse, rat and rabbit dams are sacrificed by CO_2 anesthesia prior to delivery since many animals tend to cannabilize their defective offspring. A laparotomy is performed and the uterine horns are exposed. The number of corpora lutea and number and position of live, dead, and resorbed fetuses is recorded. The umbilical cord is clamped and severed distally in order to prevent blood loss. Fetuses are removed, weighed and immediately examined by experienced personel for external anomalies as fully described by Wilson.[2]

One-half of the rodent fetuses from each litter are dissected and examined for soft tissue anomalies by the free-hand slicing method of Wilson.[2] Each fetus is fixed in 20 to 25 ml of Bouins fluid for 2 weeks. The hardened fetuses are examined for external anomalies and serially cut from the head through the trunk into 1 mm thick sections using a sharp razor blade. No slices are made beyond the kidneys and the intestines are carefully removed from the pelvic cavity. The cross sections of the fetuses and the genitourinary organs on the pelvic floor are carefully examined by experienced personnel. The remaining fetuses from each litter are processed for skeletal examination. Fetuses are fixed in 70% alcohol for 2 weeks and eviscerated. The fetuses are stored in 1% KOH for 2 days and then stained with alizarin red.[3] After differential decolorization, the skeletons are examined by experienced personnel for anomalies. For rabbits, all fetuses are examined for both soft tissue and skeletal defects.

C. Perinatal and Postnatal Study

1. Objectives

The purpose of this phase of the protocol is to determine the effect of drugs administered during the last third of pregnancy and the period of lactation. The specific areas of study are the drug effects on late fetal development, labor and delivery, lactation, neonatal viability, and growth of the newborn.

2. Method

The conditioning, mating, and establishment of pregnancy in rats and mice are as previously described. The drug is administered to the dam during the final one-third of gestation and continued throughout lactation to weaning. The test compound is incorporated into the diet and a pair-fed control group, whose food intake is limited to the least amount of food consumed by the treated group, is included in the study. Treatment in rats and mice is initiated on day 16 of gestation and continued until the pups are weaned at 21 days of age. Labor and delivery are observed whenever possible and any signs of abnormal, prolonged, or delayed labor are carefully noted. The duration of gestation is calculated for each mother in all groups. The litters are examined as soon as possible after delivery, and at 4 and 21 days of age. The examination of the pups is conducted with a minimum disturbance of the mother. The following information is recorded for all the litters in each group:

a. Litter size
b. Number of stillborn and live born
c. Anomalies of dead and live pups
d. Number and weight of pups at 4 and 21 days of age
e. Indexes of
   (1) Fertility: confirmed pregnancies/sperm positive females
   (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
   (3) Viability: pups alive at day 4/pups alive at birth
   (4) Lactation: pups alive at day 21/pup alive at day 4

III. STATISTICAL ANALYSIS OF DATA

Two important considerations in performing a valid statistical analysis are the determination of the sample size and the selection of appropriate statistical tests. In a study of developmental toxicity the sample size is determined by the selection of experimental units. The litter, rather than individual fetuses, is considered to be the unit of observation for our studies since the dam is the unit of treatment and the fetal response is dependent, to some degree, on maternal influences.

The data collected fall into two categories. The first category is enumeration or discontinuous data. Examples of discontinuous data are the number of sperm positive animals with evidence of conception, mortality, and indexes of fertility and gestation. The Fisher Exact Probability Test[4/]

is the test of choice to evaluate the significance. Such enumeration data are reported with the exact 95% confidence limits.

The second category is quantitative or continuous data. Examples of continuous data are body weight, food consumption, and the remaining indexes. Such quantitative data are reported as the mean ± the standard error (S.E.). These data are analyzed by Bartlett's test[5] for homogeneity. The tests of significance for homogeneous data are either Dunnett's procedure (one control group) or Tukey's omega procedure (more than one control group). If heterogeneity is indicated, then significance is based on multiple comparisons with the nonparametric rank test.[6] The level of significance is selected as $P < 0.05$.

IV. INTERPRETATION OF DATA

A. Phases of Fetal Development

The development of an adult organism from a single cell may be divided into six phases.[7] The first phase of development, gametogenesis, involves the growth and maturation of the egg and sperm. The gametes fuse during the second phase of development and the quiescent egg is activated to continue its developmental program. Cleavage, the third phase of development, encompasses a period of rapid cell division without a significant change in embryonic size or cellular differentiation. The embryo, at the end of cleavage, is referred to as a blastula and consists of a layer of cells, the blastoderm, surrounding a cavity, the blastocoele. The embryo attaches to the uterine wall and begins the process of placentation at the blastula stage. Gastrulation is the fourth developmental phase and involves the formation of germinal layers from the blastoderm. Primary organ rudiments are derived from the germinal layers during organogenesis, the fifth phase of development. The sixth phase of development is a period of growth and histological differentiation. The organ rudiments grow during this period and acquire the structure and biochemical properties characteristic of adult tissues. Organs grow by increasing both the number and size of cells. Tissue specific characteristics are established by a differential expression of the genetic information.

Treatments may affect the various phases of both animal and human development. The protocol used in our laboratory is designed to determine the developmental toxicity of a treatment in laboratory animals. The various parameters used to measure developmental toxicity help to determine if an agent affects any of the six developmental phases previously described. Since it is not practical to study each phase of development separately, the various phases are combined into periods of study. The units of study are the pre-implantation period (phases 1-3), post-implantation period (phases 3-5), and the period of differentiation (phase 6).

The dam and developing animal represent an integrated unit during the time of treatment. Effects which are observed in the developing animal, therefore, may be due to toxicity of the treatment in either the dam or developing animal. As development progresses it becomes more difficult to attribute an effect to a single period of study or treatment.

B. Fertility and General Reproductive Performance Study

The fertility and general reproductive performance study involves treating females during all six phases of development and treating males only during the period of gametogenesis. The effect of the treatment in females is studied at mid-gestation and after delivery. Males, on the other hand, are mated with normal females and treatment effects are studied in these females at mid-gestation and after delivery.

Some females are examined at mid-gestation and the various parameters previously described are recorded. The number of corpora lutea are counted by gross inspection and this value provides a measure of the ova released during ovulation. The number of implantations is used as a measure of the fertilized ova that developed to a stage where an attachment to the uterine wall is obvious at the time of inspection. The observations are summarized in the form of indexes. The fertility index is the percentage of mated females that are pregnant. A reduction in this index reflects pre-implantation losses. The implantation index is the percentage of ova that implant and it also provides a measure of pre-implantation losses. The implant viability index is the percentage of implants which appear normal at the time of examination. A reduction in this index serves as an indication of post-implantation losses. The gestation index is the percentage of pregnant females with one or more viable embryos and provides a measure of post-implantation survival.

Some females are examined after birth and the growth and development of the pups is recorded as previously described. Effects observed at this time may have been produced at any of the six developmental phases. The observations are summarized in the form of indexes. The fertility index provides a measure of pre-implantation losses. The gestation and implant viability indexes calculated on the basis of pups rather than embryos, provide an indication of post-implantation losses. The viability index is the percentage of live-born pups which survive to day 4. A reduction in this index reflects an effect at the post-implantation or differentation period since normal pups can survive for brief periods without maternal care. The lactation index is the percentage of pups alive on day 4 which survive to day 21 and is a measure of effects occuring during the period of treatment. A reduction in this index reflects an impaired ability of the mother to nourish the young, the passage of toxic material to the young through the milk, and/or the manifestation of a developmental defect.

Effects observed at the mid-gestation or postnatal examination in females mated with treated males are indicative of toxicity produced during spermatogenesis, the first phase of development. Abnormalities in sperm may be manifested at any of the developmental stages beginning with fertilization. The previously described parameters are used to identify these effects.

C. Teratology Study

The teratology study involves treating pregnant females during the period of organogenesis and observing fetuses prior to term in order to identify possible effects on development. Treatment of rodents from day 6 through 15 of gestation roughly corresponds to developmental stages 3 to 5 which are in the post-implantation period. If evidence of toxicity is observed during fetal examination, then a primary effect was produced at any of these stages. The primary effect may be compounded into a series of secondary effects as development progresses.

Malformations may fall into three groups.[8/] The first group is common variations and includes retarded ossifications. The second group is minor anomalies and refers to effects such as malformed sternabrae, wavy ribs, and supernumerary ribs. The third group is major malformations and includes anomalies which seriously affect the growth and survival of the offspring. Malformations are not equally significant or useful in interpreting or extrapolating animal experimental studies to man. Anomalies such as supernumerary ribs and decreased or abnormal sternal ossification patterns, for example, might be of little importance both to the animal and to attempts at predicting toxicity in humans. Malformations of doubtful significance include curly tail, straight legs, malrotated limbs and paws, wrist drop, protruding tongue, enlarged atria and/or ventricles, abnormal renal pelvic development and translucent skin.

The defects are reported as an anomaly index. The percent of the fetuses with a given defect is calculated for each litter and these values are then averaged and presented as the mean $\pm$ standard error (S.E.). The mean value provides a measure of the affected fetuses per litter for the group and the standard error provides an estimation of the distribution of the effect between litters within the group.

D. Perinatal and Postnatal Study

This study involves treating the dam during both the later portion of developmental phase 5 and most of phase 6. The growth and development of the pups is observed to monitor possible developmental toxicity. The various

indexes which are used to summarize these observations are discussed above in the section on Interpretation of the Fertility and General Reproductive Performance Study.

V. DISCUSSION OF PROTOCOL

A variety of experimental protocols are available to obtain information concerning the effects of agents on reproduction and development. An aim of these animal studies is to provide information concerning the risk of exposing the human population to chemical agents. The procedure used in our laboratory to obtain this information complies with the FDA guidelines for general reproduction, teratology, and perinatal and postnatal studies. There are problems associated with conducting and evaluating the results.

A. Problems Conducting Protocol

1. Selection of Test Animal

The ideal test animal should (1) absorb, metabolize and eliminate the test substance the same way as humans, (2) transmit the substance and its metabolites to the developing animal at the same rate as humans, and (3) have embryos, fetuses, and neonates with the same development schedules and metabolic pathways as the developing human. The existing comparative data is insufficient to determine which animal species is most like man in any of these characteristics. The currently available information, however, indicates that no presently used species, including simian primates, is like man in all of these respects.[9/] The degree of similarity to man that a given species exhibits may vary from one test substance to another. The above criteria for an ideal test animal should be considered, as far as the available information permits, in the selection of test species. The advantages and disadvantages of species commonly used for these tests are:

a. Mouse: The mouse is inexpensive to maintain in large breeding colonies and its embryology is well documented. Small size with a limited supply of tissues and body fluids is a disadvantage in the examination of fetuses for defects and in studies on absorption, metabolism, and excretion of chemical agents. Mice respond to some substances that have limited teratogenicity in other animals and have earned the reputation for unusual sensitivity to teratogens.[9/]

b. Rat: The rat has a convenient size for evaluation and analytical purposes, high fecundity, and a low incidence of spontaneous malformations. There is, however, no adequate single source of information on rat embryology, although this information is covered in numerous research papers.

c. Rabbit: The large size of this species permits the collection of large amounts of body fluids and tissues for analysis. Disease and parasites present obstacles to high reproductive performance in some laboratories and good stocks of rabbits are not universally available. The embryology is not fully documented for rabbits but is adequate for most purposes. Since this species was among the first animals to respond teratogenically to thalidomide, rabbits have been credited with greater similarity in teratogenic sensitivity to man than is warranted. There is no reason to regard the rabbit rather than the various species of rodents, which are their close relatives, as a more valid test animal for evaluating the teratogenic risk of agents in humans.[9/]

2. Selection of Dosage

Problems associated with selecting the dosage are the route, amount and duration of treatment. The practice of administering test substances to animals by the same route that will be used clinically is sound. If animals are treated orally for a short period of time, as in teratology studies, then gastric gavage is preferred to incorporating the agent into the diet. A stomach tube permits the accurate administration of a dose and eliminates the variables of food wastage and possible chemical change as a result of exposure to air, light, and other dietary ingredients. Prolonged treatment of animals by gastric gavage is not practical, however, due to the increased risk of trauma and expense associated with daily animal treatments. Agents incorporated into an animal's diet may alter the normal food intake as a result of an effect on appetite or a disagreeable odor or smell. Pair feeding, therefore, is required to determine the effect of reduced feed consumption on growth and development.

The dose levels should include a dose which produces maternal toxicity. The rationale for selecting this dose is to ensure that a maternal response is produced. Maternal toxicity may be measured in terms of lethality, weight loss or any other parameter that is related to treatment. If development is disrupted at doses which produce maternal toxicity, then lower doses should be studied in order to identify a dose below which no effect is observed on development. The identification of a dose which produces neither adult nor developmental toxicity is of value in estimating a safe dose for humans.

Animals are treated throughout various phases of development in this protocol to determine the effect of the agent on development. A treatment schedule which involves prolonged drug exposure presents three basic problems which affect the actual level of drug exposure and the detection of developmental toxicity. First, prolonged drug exposure may increase the activity of the drug metabolizing enzymes which are responsible for the biotransformation of chemicals. The metabolism of the test compound, therefore, is increased; maternal blood levels of the parent compound are decreased;

and maternal exposure to metabolites may be increased. Second, prolonged drug exposure may produce liver and/or kidney damage. A reduction in the functional capability of the liver reduces the biotransformation of the test compound while impaired kidney function may reduce the elimination of the drug from the body. Third, if a compound is administered during the early portion of gestation, then implantation and early embryonic survival may be impaired. The presence of small litter size and a high degree of resorption prevents the detection of teratogenic effects.

The length of gestation in most experimental animals is short compared to that of humans. Treating experimental animals during gestation may not produce tissue levels which could occur from more prolonged drug exposure as in human pregnancy. This difficulty can, in some cases, be overcome by increasing the dose, but problems may arise if the drug is poorly absorbed or degraded prior to absorption.

3. Determination of Feed Consumption

Animals may be treated during developmental toxicity studies by incorporating the test compound(s) into their diet. The compounds may represent either a fixed or variable percent of the diet. Since feed consumption varies during gestation and lactation, it is advisable to administer the drug as a variable percent of the diet in order to administer a constant amount of drug. The drug intake can be calculated from the percent of the drug in the diet and the amount of feed consumed. Thus, an accurate estimation of feed intake is imperative.

Accurate measurement of feed intake in laboratory animals, especially in rodents, is difficult due to spillage. Feed can be given to rats in stainless steel diet feeders (Model HB-69, Hoeltge, Cincinnati, Ohio) and to mice in stainless steel compartment feeders (Lab Products Inc., Garfield, New Jersey) which are designed to eliminate spillage. In most cases, these feeders are spill-proof; however, animals occasionally acquire the necessary skill to defeat the feeder. When feed consumption is high and the spillage can be measured, then the true feed consumption is calculated. If, on the other hand, the spillage can not be reasonably estimated, the result is omitted.

B. Problems Interpreting the Data

The ultimate goal of testing drugs in animals is to obtain information for making predictive statements concerning a drug's effect in humans. There are problems inherent to animal experiments, particularly in reproduction and teratology studies, which make this extrapolation especially difficult. After the data from a developmental toxicity study have been collected

and analyzed statistically, it is necessary to determine both the significance of defects on normal adult animals and the relevance of the defects to humans.

When developing animals are examined at various times after treatment, evidence of deviant development, as demonstrated by growth retardation, malformations, intrauterine death and functional defects, may be apparent. These observations, however, do not provide information concerning the consequences of these effects in the adult. Growth retardation, for example, may be present in fetuses during a teratology study but may be absent in the adult as a result of maturation and compensatory growth processes. A delayed ossification of bones and the presence of extra ribs are examples of defects which may be corrected during growth or present a problem of questionable significance to the adult. The relevance of these defects to normal growth are, in some cases, difficult to assess experimentally.

The variation between species in response to agents presents the major obstacle to achieving the ultimate goal of any drug testing program. These unique responses of species to agents may be due to metabolic and pharmacokinetic factors.[10] The complexity of the animal system and degree of interspecies variability increases during development as a result of the formation of a placenta and its influence on drug transport, and a changing embryonic sensitivity to drugs. The ability to demonstrate developmental toxicity, therefore, depends on biotransformation of the drug by the mother, placenta, or embryo; pharmacokinetic properties of the drug in the mother and embryo; and embryonic sensitivity at the time of treatment.

VI. TERMINOLOGY OF ANOMALIES

A. Gross Anomalies

1. General

edematous - abnormal accumulation of clear fluid under the skin
hematoma - a localized mass of extravasated blood that is relatively or completely confined within an organ or tissue: not as a result of cesarean section handling
immature skin - skin is sticky with a shiny appearance

2. Head

anophthalmia - absence of one or both eyes
brachygnathia - abnormal shortness or recession of the mandibles
cranium, domed - excessively domed cranium suggestive of hydrocephalus

exencephalus - skull defective, the brain is exposed or extruded
eye, open - eyeball exposed with lids absent or withdrawn
lip, cleft - fissure in the lip, usually causing conjunction of nasal passage and mouth
meningocele - skin intact, translucent, and elevated by a fluid filled vesicle of meninges which protrude through a midline defect in the cranium
meningoencephalocele - meninges and part of brain protruding through a cranial defect to cause an irregular mass beneath the skin
microphthalmia - small or rudimentary eyes
palate, cleft - fissure in hard palate, due to a failure of the palatine shelves to unite
platycephaly - flatness of the skull

3. Trunk

anus, closed - (imperforate anus) anus closed by a membrane so as to prevent the normal passage of intestinal contents
gastroschisis - protrusion of intestines and other abdominal viscera through a ventral midline defect
kyphosis - convexity backward, dorsal-ventral curvature of the spine
myelomeningocele - absence of the vertebral arches through which the spinal cord and its membranes protrude, denoted by a bubble-like bulge along the dorsal midline
rhachischisis - congenital fissure of the spinal column with failure of the skin and vertebral column to close
spina bifida - absence of the vertebral arches through which the spinal membranes, with or without the spinal-cord tissue, protrude, denoted by a raw, usually bloody depression
umbilical hernia - protrusion of intestines through a small ventral midline defect

4. Extremities

acaudate - no tail
adactyly - absence of digits
club foot - abnormal flexion of the foot
micromelia - rudimentary limbs
oligodactyly - fewer than five digits
polydactyly - more than five digits
syndactyly - fused or webbed digits
tail, short - tail is less than half the normal length

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APPENDIX III

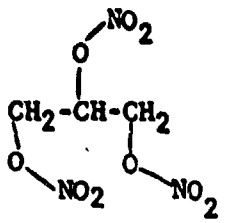
TNG ASSAYS

MIDWEST RESEARCH INSTITUTE
CONTRACT NO. DAMD-17-74-C-4073
7 July 1975

Data on: Trinitroglycerin

Supplier: Atlas Chemical Division
of ICI America Inc.

Lot No.: D17-H3



10% trinitroglycerin on lactose

I. Identity

The sample was extracted with chloroform to remove the trinitroglycerin. Evaporation of the chloroform gave a liquid whose infrared spectrum (between salt plates) was identical to that reported for trinitroglycerin.[1/]

II. Assay

A. Gas Chromatography

The sample was studied by gas chromatography using the following system:

1. Instrument: Bendix 2500 equipped with flame ionization detector
2. Column: glass, 6 ft x 1/4 in
   1.5% DC LSX-3-0295
   1.5% GE-XE-60
   on Gas Chrom Q
3. Nitrogen flow: 30 cc/min
4. Detector T°: 200°
5. Injector T°: 130°
6. Column T°: 130°

1/ Alma L. Hayden, Oscar R. Sammul, George B. Selzer, and Jonas Carol, "Infrared and Ultraviolet Spectra of Some Compounds of Pharmaceutical Interest," Association of Offical Analytical Chemists, Washington, 1972, p. 150.

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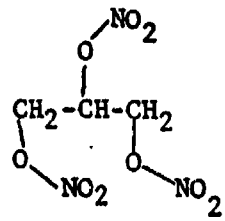

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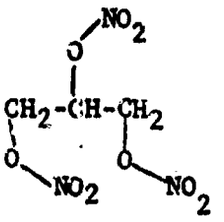

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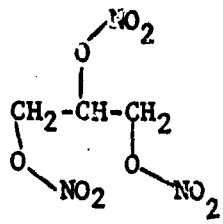

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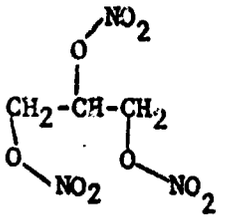

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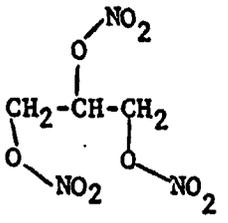

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8. Results: With the peak for trinitroglycerin representing 100%, no other peaks were observed at 1% concentration level by peak area comparison.

B. Nitro Content

The Lot was analyzed for nitroglycerin content by the method of Wells.[2/] The Lot contains 9.72 ± 0.09% trinitroglycerin.

III. Conclusions

Lot D17-H3 contains 9.72 ± 0.09% trinitroglycerin.

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Junior Chemist

Approved:

Danny O. Helton
Associate Chemist

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7. References: 1-mononitroglycerin, 2-mononitroglycerin, 1,2-dinitroglycerin, 1,3-dinitroglycerin, U.S.P. trinitroglycerin

8. Results: With the peak for trinitroglycerin representing 100%, no other peaks were observed at 1% concentration level by peak area comparison.

B. Nitro Content

The Lot was analyzed for nitroglycerin content by the method of Wells.[2/] The Lot contains 9.72 ± 0.09% trinitroglycerin.

III. Conclusions

Lot D17-H3 contains 9.72 ± 0.09% trinitroglycerin.

MIDWEST RESEARCH INSTITUTE

John Rollheiser
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2/ Clyde E. Wells, Harvey M. Miller, and Yvonne H. Pfabe, Journal of the Association of Official Analytical Chemists, 53, 579 (1970).

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